



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C12N 5/14, 15/00, 15/05, 15/09, 15/29, 15/31, 15/64, 15/82, A01G 13/00, A01H 1/00</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 97/17432</b> <b>(43) International Publication Date:</b> 15 May 1997 (15.05.97)
<b>(21) International Application Number:</b> PCT/US96/18003 <b>(22) International Filing Date:</b> 6 November 1996 (06.11.96)  <b>(30) Priority Data:</b> 60/007,255           6 November 1995 (06.11.95)   US 08/608,423           28 February 1996 (28.02.96)   US 08/705,484           28 August 1996 (28.08.96)   US  <b>(71) Applicant:</b> WISCONSIN ALUMNI RESEARCH FOUNDATION [US/US]; 614 North Walnut Street, Madison, WI 53707-7365 (US).		<b>(72) Inventors:</b> ENSIGN, Jerald, C.; 1810 North Walnut Street, Madison, WI 53705 (US). BOWEN, David, J.; 5668 Highway A, Oregon, WI 53575 (US). PETELL, James; 1427 Hunters Glen, Zionsville, IN 46077 (US). FATIG, Raymond; 30 Clay Court, Zionsville, IN 46077 (US). SCHOONOVER, Sue; 7142 Marstella, Brownsburg, IN 46112 (US). FFRENCH-CONSTANT, Richard, H.; 1006 University Bay Drive, Madison, WI 53705 (US). ROCHE-LEAU, Thomas, A.; 3100 Buena Vista Street, Madison, WI 53704 (US). BLACKBURN, Michael, B.; 2127 Lunn Lane, Madison, WI 53713 (US). HEY, Timothy, D.; 1653 Catalina Way, Zionsville, IN 46077 (US). MERLO, Donald, J.; 11845 Durbin Drive, Carmel, IN 46032 (US). ORR, Gregory, L.; 1028 Saratoga Circle, Indianapolis, IN 46280 (US). ROBERTS, Jean, L.; 26035 State Road 19, Arcadia, IN 46030 (US). STRICKLAND, James, A.; 780 Mt. Zion Road, Lebanon, IN 46052 (US). GUO, Lining; 7 Nelson Circle, Brownsburg, IN 46112 (US). CICHE, Todd, A.; 1609 Chadbourne Avenue, Madison, WI 53705 (US).  <b>(74) Agent:</b> SEAY, Nicholas, J.; Quarles & Brady, P.O. Box 2113, Madison, WI 53701-2113 (US).  <b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> INSECTICIDAL PROTEIN TOXINS FROM PHOTORHABDUS  <b>(57) Abstract</b>  Proteins from the genus <i>Photorhabdus</i> are toxic to insects upon exposure. <i>Photorhabdus luminescens</i> (formerly <i>Xenorhabdus luminescens</i> ) have been found in mammalian clinical samples and as a bacterial symbiont of entomopathogenic nematodes of genus <i>Heterorhabditis</i> . These protein toxins can be applied to, or genetically engineered into, insect larvae food and plants for insect control.		

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INSECTICIDAL PROTEIN TOXINS FROM *PHOTORHABDUS*

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Field of the Invention

The present invention relates to toxins isolated from bacteria and the use of said toxins as insecticides.

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Background of the Invention

Many insects are widely regarded as pests to homeowners, to picnickers, to gardeners, and to farmers and others whose  
25 investments in agricultural products are often destroyed or diminished as a result of insect damage to field crops. Particularly in areas where the growing season is short, significant insect damage can mean the loss of all profits to growers and a dramatic decrease in crop yield. Scarce supply of  
30 particular agricultural products invariably results in higher costs to food processors and, then, to the ultimate consumers of food plants and products derived from those plants.

Preventing insect damage to crops and flowers and eliminating the nuisance of insect pests have typically relied on  
35 strong organic pesticides and insecticides with broad toxicities. These synthetic products have come under attack by the general population as being too harsh on the environment and on those exposed to such agents. Similarly in non-agricultural settings, homeowners would be satisfied to have insects avoid their homes  
40 or outdoor meals without needing to kill the insects.

The extensive use of chemical insecticides has raised environmental and health concerns for farmers, companies that produce the insecticides, government agencies, public interest groups, and the public in general. The development of less  
5 intrusive pest management strategies has been spurred along both by societal concern for the environment and by the development of biological tools which exploit mechanisms of insect management. Biological control agents present a promising alternative to chemical insecticides.

10 Organisms at every evolutionary development level have devised means to enhance their own success and survival. The use of biological molecules as tools of defense and aggression is known throughout the animal and plant kingdoms. In addition, the relatively new tools of the genetic engineer allow modifications  
15 to biological insecticides to accomplish particular solutions to particular problems.

One such agent, *Bacillus thuringiensis* (Bt), is an effective insecticidal agent, and is widely commercially used as such. In fact, the insecticidal agent of the Bt bacterium is a protein  
20 which has such limited toxicity, it can be used on human food crops on the day of harvest. To non-targeted organisms, the Bt toxin is a digestible non-toxic protein.

Another known class of biological insect control agents are certain genera of nematodes known to be vectors of transmission  
25 for insect-killing bacterial symbionts. Nematodes containing insecticidal bacteria invade insect larvae. The bacteria then kill the larvae. The nematodes reproduce in the larval cadaver. The nematode progeny then eat the cadaver from within. The bacteria-containing nematode progeny thus produced can then  
30 invade additional larvae.

In the past, insecticidal nematodes in the *Steinernema* and *Heterorhabditis* genera were used as insect control agents. Apparently, each genus of nematode hosts a particular species of bacterium. In nematodes of the *Heterorhabditis* genus, the  
35 symbiotic bacterium is *Photorhabdus luminescens*.

Although these nematodes are effective insect control agents, it is presently difficult, expensive, and inefficient to produce, maintain, and distribute nematodes for insect control.

It has been known in the art that one may isolate an  
40 insecticidal toxin from *Photorhabdus luminescens* that has



activity only when injected into Lepidopteran and Coleopteran insect larvae. This has made it impossible to effectively exploit the insecticidal properties of the nematode or its bacterial symbiont. What would be useful would be a more practical, less labor-intensive wide-area delivery method of an insecticidal toxin which would retain its biological properties after delivery. It would be quite desirable to discover toxins with oral activity produced by the genus *Photorhabdus*. The isolation and use of these toxins are desirable due to efficacious reasons. Until applicants' discoveries, these toxins had not been isolated or characterized.

#### Summary of the Invention

The native toxins are protein complexes that are produced and secreted by growing bacteria cells of the genus *Photorhabdus*. of interest are the proteins produced by the species *Photorhabdus luminescens*. The protein complexes, with a molecular size of approximately 1,000 kDa, can be separated by SDS-PAGE gel analysis into numerous component proteins. The toxins contain no hemolysin, lipase, type C phospholipase, or nuclease activities. The toxins exhibit significant toxicity upon exposure administration to a number of insects.

The present invention provides an easily administered insecticidal protein as well as the expression of toxin in a heterologous system.

The present invention also provides a method for delivering insecticidal toxins that are functional active and effective against many orders of insects.

Objects, advantages, and features of the present invention will become apparent from the following specification.

#### Brief Description of the Drawings

Fig. 1 is an illustration of a match of cloned DNA isolates used as a part of sequence genes for the toxin of the present invention.

Fig. 2 is a map of three plasmids used in the sequencing process.

Fig. 3 is a map illustrating the inter-relationship of several partial DNA fragments.

Fig. 4 is an illustration of a homology analysis between the protein sequences of TcbA<sub>ii</sub> and TcaB<sub>ii</sub> proteins.

5 Fig. 5 is a phenogram of *Photorhabdus* strains. Relationship of *Photorhabdus* Strains was defined by rep-PCR.

The upper axis of Fig. 5 measures the percentage similarity of strains based on scoring of rep-PCR products (i.e., 0.0 [no similarity] to 1.0 [100% similarity]). At the right axis, the  
10 numbers and letters indicate the various strains tested; 14=W-14, Hm=Hm, H9=H9, 7=WX-7, 1=WX-1, 2=WX-2, 88=HP88, NC-1=NC-1, 4=WX-4, 9=WX-9, 8=WX-8, 10=WX-10, WIR=WIR, 3=WX-3, 11=WX-11, 5=WX-5, 6=WX-6, 12=WX-12, x14=WX-14, 15=WX-15, Hb=Hb, B2=B2, 48 through 52=ATCC 43948 through ATCC 43952. Vertical lines separating  
15 horizontal lines indicate the degree of relatedness (as read from the extrapolated intersection of the vertical line with the upper axis) between strains or groups of strains at the base of the horizontal lines (e.g., strain W-14 is approximately 60% similar to strains H9 and Hm).

20 Fig. 6 is an illustration of the genomic maps of the W-14 Strain.

#### Detailed Description of the Invention

25 The present inventions are directed to the discovery of a unique class of insecticidal protein toxins from the genus *Photorhabdus* that have oral toxicity against insects. A unique feature of *Photorhabdus* is its bioluminescence. *Photorhabdus* may be isolated from a variety of sources. One such source is  
30 nematodes, more particularly nematodes of the genus *Heterorhabditis*. Another such source is from human clinical samples from wounds, see Farmer et al. 1989 J. Clin. Microbiol. 27 pp. 1594-1600. These saprophytic strains are deposited in the American Type Culture Collection (Rockville, MD) ATCC #s 43948,  
35 43949, 43950, 43951, and 43952, and are incorporated herein by reference. It is possible that other sources could harbor *Photorhabdus* bacteria that produce insecticidal toxins. Such sources in the environment could be either terrestrial or aquatic based.

The genus *Photorhabdus* is taxonomically defined as a member of the Family *Enterobacteriaceae*, although it has certain traits atypical of this family. For example, strains of this genus are nitrate reduction negative, yellow and red pigment producing and bioluminescent. This latter trait is otherwise unknown within the *Enterobacteriaceae*. *Photorhabdus* has only recently been described as a genus separate from the *Xenorhabdus* (Boemare et al., 1993 Int. J. Syst. Bacteriol. 43, 249-255). This differentiation is based on DNA-DNA hybridization studies, phenotypic differences (e.g., presence (*Photorhabdus*) or absence (*Xenorhabdus*) of catalase and bioluminescence) and the Family of the nematode host (*Xenorhabdus*; *Steinernematidae*, *Photorhabdus*; *Heterorhabditidae*). Comparative, cellular fatty-acid analyses (Janse et al. 1990, Lett. Appl. Microbiol 10, 131-135; Suzuki et al. 1990, J. Gen. Appl. Microbiol., 36, 393-401) support the separation of *Photorhabdus* from *Xenorhabdus*.

In order to establish that the strain collection disclosed herein was comprised of *Photorhabdus* strains, the strains were characterized based on recognized traits which define *Photorhabdus* and differentiate it from other *Enterobacteriaceae* and *Xenorhabdus* species. (Farmer, 1984 Bergey's Manual of Systemic Bacteriology Vol. 1 pp.510-511; Akhurst and Boemare 1988, J. Gen. Microbiol. 134 pp.1835-1845; Boemare et al. 1993 Int. J. Syst. Bacteriol. 43 pp.249-255, which are incorporated herein by reference). The traits studied were the following: gram stain negative rods, organism size, colony pigmentation, inclusion bodies, presence of catalase, ability to reduce nitrate, bioluminescence, dye uptake, gelatin hydrolysis, growth on selective media, growth temperature, survival under anerobic conditions and motility. Fatty acid analysis was used to confirm that the strains herein all belong to the single genus *Photorhabdus*.

Currently, the bacterial genus *Photorhabdus* is comprised of a single defined species, *Photorhabdus luminescens* (ATCC Type strain #29999, Poinar et al., 1977, Nematologica 23, 97-102). A variety of related strains have been described in the literature (e.g. Akhurst et al. 1988 J. Gen. Microbiol., 134, 1835-1845; Boemare et al. 1993 Int. J. Syst. Bacteriol. 43 pp. 249-255; Putz et al. 1990, Appl. Environ. Microbiol., 56, 181-186). Numerous

*Photorhabdus* strains have been characterized herein. Such strains are listed in Table 18 in the Examples. Because there is currently only one species (*luminescens*) defined within the genus *Photorhabdus*, the *luminescens* species traits were used to  
5 characterize the strains herein. As can be seen in Fig. 5, these strains are quite diverse. It is not unforeseen that in the future there may be other *Photorhabdus* species that will have some of the attributes of the *luminescens* species as well as some different characteristics that are presently not defined as a  
10 trait of *Photorhabdus luminescens*. However, the scope of the invention herein is to any *Photorhabdus* species or strains which produce proteins that have functional activity as insect control agents, regardless of other traits and characteristics.

Furthermore, as is demonstrated herein, the bacteria of the  
15 genus *Photorhabdus* produce proteins that have functional activity as defined herein. Of particular interest are proteins produced by the species *Photorhabdus luminescens*. The inventions herein should in no way be limited to the strains which are disclosed herein. These strains illustrate for the first time that  
20 proteins produced by diverse isolates of *Photorhabdus* are toxic upon exposure to insects. Thus, included within the inventions described herein are the strains specified herein and any mutants thereof, as well as any strains or species of the genus  
*Photorhabdus* that have the functional activity described herein.

25 There are several terms that are used herein that have a particular meaning and are as follows:

By "functional activity" it is meant herein that the protein toxins function as insect control agents in that the proteins are  
30 orally active, or have a toxic effect, or are able to disrupt or deter feeding, which may or may not cause death of the insect. When an insect comes into contact with an effective amount of toxin delivered via transgenic plant expression, formulated protein compositions(s), sprayable protein composition(s), a bait  
35 matrix or other delivery system, the results are typically death of the insect, or the insects do not feed upon the source which makes the toxins available to the insects.

The protein toxins discussed herein are typically referred to as "insecticides". By insecticides it is meant herein that the protein toxins have a "functional activity" as further defined herein and are used as insect control agents.

5

By the use of the term "oligonucleotides" it is meant a macromolecule consisting of a short chain of nucleotides of either RNA or DNA. Such length could be at least one nucleotide, but typically are in the range of about 10 to about 12

10

nucleotides. The determination of the length of the oligonucleotide is well within the skill of an artisan and should not be a limitation herein. Therefore, oligonucleotides may be less than 10 or greater than 12.

15

By the use of the term "toxic" or "toxicity" as used herein it is meant that the toxins produced by *Photorhabdus* have "functional activity" as defined herein.

20

By the use of the term "genetic material" herein, it is meant to include all genes, nucleic acid, DNA and RNA.

25

Fermentation broths from selected strains reported in Table 18 were used to determine the following: breadth of insecticidal toxin production by the *Photorhabdus* genus, the insecticidal spectrum of these toxins, and to provide source material to purify the toxin complexes. The strains characterized herein have been shown to have oral toxicity against a variety of insect orders. Such insect orders include but are not limited to *Coleoptera*, *Homoptera*, *Lepidoptera*, *Diptera*, *Acarina*, *Hymenoptera* and *Dictyoptera*.

30

As with other bacterial toxins, the rate of mutation of the bacteria in a population causes many related toxins slightly different in sequence to exist. Toxins of interest here are those which produce protein complexes toxic to a variety of insects upon exposure, as described herein. Preferably, the toxins are active against *Lepidoptera*, *Coleoptera*, *Homoptera*, *Diptera*, *Hymenoptera*, *Dictyoptera* and *Acarina*. The inventions herein are intended to capture the protein toxins homologous to protein toxins produced by the strains herein and any derivative

40

5 By the use of the term "*Photorhabdus* toxin" it is meant any  
protein produced by a *Photorhabdus* microorganism strain  
which has functional activity against insects, where the  
*Photorhabdus* toxin could be formulated as a sprayable  
composition, expressed by a transgenic plant, formulated as  
10 a bait matrix, delivered via a Baculovirus, or delivered by  
any other applicable host or delivery system.

strains thereof, as well as any protein toxins produced by *Photorhabdus*. These homologous proteins may differ in sequence, but do not differ in function from those toxins described herein. Homologous toxins are meant to include protein complexes of  
5 between 300 kDa to 2,000 kDa and are comprised of at least two (2) subunits, where a subunit is a peptide which may or may not be the same as the other subunit. Various protein subunits have been identified and are taught in the Examples herein. Typically, the protein subunits are between about 18 kDa to about  
10 230 kDa; between about 160 kDa to about 230 kDa; 100 kDa to 160 kDa; about 80 kDa to about 100 kDa; and about 50 kDa to about 80 kDa.

As discussed above, some *Photorhabdus* strains can be isolated from nematodes. Some nematodes, elongated cylindrical  
15 parasitic worms of the phylum *Nematoda*, have evolved an ability to exploit insect larvae as a favored growth environment. The insect larvae provide a source of food for growing nematodes and an environment in which to reproduce. One dramatic effect that follows invasion of larvae by certain nematodes is larval death.  
20 Larval death results from the presence of, in certain nematodes, bacteria that produce an insecticidal toxin which arrests larval growth and inhibits feeding activity.

Interestingly, it appears that each genus of insect parasitic nematode hosts a particular species of bacterium,  
25 uniquely adapted for symbiotic growth with that nematode. In the interim since this research was initiated, the name of the bacterial genus *Xenorhabdus* was reclassified into the *Xenorhabdus* and the *Photorhabdus*. Bacteria of the genus *Photorhabdus* are characterized as being symbionts of *Heterorhabditus* nematodes  
30 while *Xenorhabdus* species are symbionts of the *Steinernema* species. This change in nomenclature is reflected in this specification, but in no way should a change in nomenclature alter the scope of the inventions described herein.

The peptides and genes that are disclosed herein are named  
35 according to the guidelines recently published in the Journal of Bacteriology "Instructions to Authors" p. i-xii (Jan. 1996), which is incorporated herein by reference. The following peptides and genes were isolated from *Photorhabdus* strain W-14.

Peptide / Gene Nomenclature  
Toxin complex (Tc)

5	Peptide Name	Gene Name	Patent Sequence ID#
	<u>tca genomic region</u>		
	TcaA	tcaA	12
	TcaAiii	tcaA	4
10	TcaBi	tcaB	3 (19, 20)
	TcaBii	tcaB	5
	TcaC	tcaC	2
	<u>tcb genomic region</u>		
15	TcbA	tcbA	16
	TcbAi	tcbA	(pro-peptide)
	TcbAii	tcbA	1 (21, 22, 23, 24)
	TcbAiii	tcbA	40
20	<u>tcc genomic region</u>		
	TccA	tccA	8
	TccB	tccB	7
	<u>tcd genomic region</u>		
25	TcdAi	tcdA	(pro-peptide)
	TcdAii	tcdA	13, (38, 39)
			17, 18)
	TcdAiii	tcdA	41, (42, 43)
	TcdB	tcdB	14

(bracket sequence indicates internal amino acid sequence obtained by tryptic digests)

35       The sequences listed above are grouped by genomic region. The *tcbA* gene was expressed in *E. coli* as two protein fragments TcbA and TcbAiii as illustrated in the Examples. It may be beneficial to have proteolytic clippage of some sequences to obtain the higher activity of the toxins for commercial

40 transgenic applications.

      The toxins described herein are quite unique in that the toxins have functional activity, which is key to developing an insect management strategy. In developing an insect management

45 strategy, it is possible to delay or circumvent the protein degradation process by injecting a protein directly into an organism, avoiding its digestive tract. In such cases, the protein administered to the organism will retain its function until it is denatured, non-specifically degraded, or eliminated

50 by the immune system in higher organisms. Injection into insects



of an insecticidal toxin has potential application only in the laboratory, and then only on large insects which are easily injected. The observation that the insecticidal protein toxins herein described exhibits their toxic activity after oral

5 ingestion or contact with the toxins permits the development of an insect management plan based solely on the ability to incorporate the protein toxins into the insect diet. Such a plan could result in the production of insect baits.

The *Photorhabdus* toxins may be administered to insects in a  
10 purified form. The toxins may also be delivered in amounts from about 1 to about 100 mg / liter of broth. This may vary upon formulation condition, conditions of the inoculum source, techniques for isolation of the toxin, and the like. The toxins may be administered as an exudate secretion or cellular protein  
15 originally expressed in a heterologous prokaryotic or eukaryotic host. Bacteria are typically the hosts in which proteins are expressed. Eukaryotic hosts could include but are not limited to plants, insects and yeast. Alternatively, the toxins may be produced in bacteria or transgenic plants in the field or in the  
20 insect by a baculovirus vector. Typically the toxins will be introduced to the insect by incorporating one or more of the toxins into the insects' feed.

Complete lethality to feeding insects is useful but is not required to achieve useful toxicity. If the insects avoid the  
25 toxin or cease feeding, that avoidance will be useful in some applications, even if the effects are sublethal. For example, if insect resistant transgenic crop plants are desired, a reluctance of insects to feed on the plants is as useful as lethal toxicity to the insects since the ultimate objective is protection of the  
30 plants rather than killing the insect.

There are many other ways in which toxins can be incorporated into an insect's diet. As an example, it is possible to adulterate the larval food source with the toxic protein by spraying the food with a protein solution, as  
35 disclosed herein. Alternatively, the purified protein could be genetically engineered into an otherwise harmless bacterium, which could then be grown in culture, and either applied to the food source or allowed to reside in the soil in an area in which insect eradication was desirable. Also, the protein could be  
40 genetically engineered directly into an insect food source. For

instance, the major food source of many insect larvae is plant material.

By incorporating genetic material that encodes the insecticidal properties of the *Photographus* toxins into the genome of a plant eaten by a particular insect pest, the adult or larvae would die after consuming the food plant. Numerous members of the monocotyledonous and dictyledenous genera have been transformed. Transgenic agronomic crops as well as fruits and vegetables are of commercial interest. Such crops include but are not limited to maize, rice, soybeans, canola, sunflower, alfalfa, sorghum, wheat, cotton, peanuts, tomatoes, potatoes, and the like. Several techniques exist for introducing foreign genetic material into plant cells, and for obtaining plants that stably maintain and express the introduced gene. Such techniques include acceleration of genetic material coated onto microparticles directly into cells (U.S. Patents 4,945,050 to Cornell and 5,141,131 to DowElanco). Plants may be transformed using *Agrobacterium* technology, see U.S. Patent 5,177,010 to University of Toledo, 5,104,310 to Texas A&M, European Patent Application 0131624B1, European Patent Applications 120516, 159418B1 and 176,112 to Schilperoot, U.S. Patents 5,149,645, 5,469,976, 5,464,763 and 4,940,838 and 4,693,976 to Schilperoot, European Patent Applications 116718, 290799, 320500 all to MaxPlanck, European Patent Applications 604662 and 627752 to Japan Tobacco, European Patent Applications 0267159, and 0292435 and U.S. Patent 5,231,019 all to Ciba Geigy, U.S. Patents 5,463,174 and 4,762,785 both to Calgene, and U.S. Patents 5,004,863 and 5,159,135 both to Agracetus. Other transformation technology includes whiskers technology, see U.S. Patents 5,302,523 and 5,464,765 both to Zeneca. Electroporation technology has also been used to transform plants, see WO 87/06614 to Boyce Thompson Institute, 5,472,869 and 5,384,253 both to Dekalb, WO9209696 and WO9321335 both to PGS. All of these transformation patents and publications are incorporated by reference. In addition to numerous technologies for transforming plants, the type of tissue which is contacted with the foreign genes may vary as well. Such tissue would include but would not be limited to embryogenic tissue, callus tissue type I and II, hypocotyl, meristem, and the like. Almost all plant tissues may

be transformed during dedifferentiation using appropriate techniques within the skill of an artisan.

Another variable is the choice of a selectable marker. The preference for a particular marker is at the discretion of the artisan, but any of the following selectable markers may be used along with any other gene not listed herein which could function as a selectable marker. Such selectable markers include but are not limited to aminoglycoside phosphotransferase gene of transposon Tn5 (Aph II) which encodes resistance to the antibiotics kanamycin, neomycin and G418, as well as those genes which code for resistance or tolerance to glyphosate; hygromycin; methotrexate; phosphinothricin (bialophos); imidazolinones, sulfonylureas and triazolopyrimidine herbicides, such as chlorosulfuron; bromoxynil, dalapon and the like.

In addition to a selectable marker, it may be desirable to use a reporter gene. In some instances a reporter gene may be used without a selectable marker. Reporter genes are genes which are typically not present or expressed in the recipient organism or tissue. The reporter gene typically encodes for a protein which provides for some phenotypic change or enzymatic property. Examples of such genes are provided in K. Weising et al. Ann. Rev. Genetics, 22, 421 (1988), which is incorporated herein by reference. A preferred reporter gene is the glucuronidase (GUS) gene.

Regardless of transformation technique, the gene is preferably incorporated into a gene transfer vector adapted to express the *Photobacterium* toxins in the plant cell by including in the vector a plant promoter. In addition to plant promoters, promoters from a variety of sources can be used efficiently in plant cells to express foreign genes. For example, promoters of bacterial origin, such as the octopine synthase promoter, the nopaline synthase promoter, the mannopine synthase promoter; promoters of viral origin, such as the cauliflower mosaic virus (35S and 19S) and the like may be used. Plant promoters include, but are not limited to ribulose-1,6-bisphosphate (RUBP) carboxylase small subunit (ssu), beta-conglycinin promoter, phaseolin promoter, ADH promoter, heat-shock promoters and tissue specific promoters. Promoters may also contain certain enhancer sequence elements that may improve the transcription efficiency. Typical enhancers include but are not limited to Adh-intron 1 and

Adh-intron 6. Constitutive promoters may be used. Constitutive promoters direct continuous gene expression in all cells types and at all times (e.g., actin, ubiquitin, CaMV 35S). Tissue specific promoters are responsible for gene expression in  
5 specific cell or tissue types, such as the leaves or seeds (e.g., zein, oleosin, napin, ACP) and these promoters may also be used. Promoters may also be are active during a certain stage of the plants' development as well as active in plant tissues and organs. Examples of such promoters include but are not limited  
10 to pollen-specific, embryo specific, corn silk specific, cotton fiber specific, root specific, seed endosperm specific promoters and the like.

Under certain circumstances it may be desirable to use an inducible promoter. An inducible promoter is responsible for  
15 expression of genes in response to a specific signal, such as: physical stimulus (heat shock genes); light (RUBP carboxylase); hormone (Em); metabolites; and stress. Other desirable transcription and translation elements that function in plants may be used. Numerous plant-specific gene transfer vectors are  
20 known to the art.

In addition, it is known that to obtain high expression of bacterial genes in plants it is preferred to reengineer the bacterial genes so that they are more efficiently expressed in the cytoplasm of plants. Maize is one such plant where it is  
25 preferred to reengineer the bacterial gene(s) prior to transformation to increase the expression level of the toxin in the plant. One reason for the reengineering is the very low G+C content of the native bacterial gene(s) (and consequent skewing towards high A+T content). This results in the generation of  
30 sequences mimicking or duplicating plant gene control sequences that are known to be highly A+T rich. The presence of some A+T-rich sequences within the DNA of the gene(s) introduced into plants (e.g., TATA box regions normally found in gene promoters) may result in aberrant transcription of the gene(s). On the  
35 other hand, the presence of other regulatory sequences residing in the transcribed mRNA (e.g., polyadenylation signal sequences (AAUAAA), or sequences complementary to small nuclear RNAs involved in pre-mRNA splicing) may lead to RNA instability. Therefore, one goal in the design of reengineered bacterial

gene(s), more preferably referred to as plant optimized gene(s), is to generate a DNA sequence having a higher G+C content, and preferably one close to that of plant genes coding for metabolic enzymes. Another goal in the design of the plant optimized

5 gene(s) is to generate a DNA sequence that not only has a higher G+C content, but by modifying the sequence changes, should be made so as to not hinder translation.

An example of a plant that has a high G+C content is maize. The table below illustrates how high the G+C content is in maize.

10 As in maize, it is thought that G+C content in other plants is also high.

Table 1  
Compilation of G+C contents of protein coding regions  
of maize genes

15

Protein Class <sup>a</sup>	Range %G+C	Mean %G+C <sup>b</sup>
Metabolic Enzymes (40)	44.4-75.3	59.0 (8.0)
Storage Proteins		
Group I (23)	46.0-51.9	48.1 (1.3)
Group II (13)	60.4-74.3	67.5 (3.2)
Group I + II (36)	46.0-74.3	55.1 (9.6) <sup>c</sup>
Structural Proteins (18)	48.6-70.5	63.6 (6.7)
Regulatory Proteins (5)	57.2-68.9	62.0 (4.9)
Uncharacterized Proteins (9)	41.5-70.3	64.3 (7.2)
<b>All Proteins (108)</b>	<b>44.4-75.3</b>	<b>60.8 (5.2)</b>

<sup>a</sup> Number of genes in class given in parentheses.

<sup>b</sup> Standard deviations given in parentheses.

<sup>c</sup> Combined groups mean ignored in calculation of overall mean.

20 For the data in Table 1, coding regions of the genes were extracted from GenBank (Release 71) entries, and base compositions were calculated using the MacVector™ program (IBI, New Haven, CT). Intron sequences were ignored in the

calculations. Group I and II storage protein gene sequences were distinguished by their marked difference in base composition.

Due to the plasticity afforded by the redundancy of the genetic code (i.e., some amino acids are specified by more than one codon), evolution of the genomes of different organisms or classes of organisms has resulted in differential usage of redundant codons. This "codon bias" is reflected in the mean base composition of protein coding regions. For example, organisms with relatively low G+C contents utilize codons having A or T in the third position of redundant codons, whereas those having higher G+C contents utilize codons having G or C in the third position. It is thought that the presence of "minor" codons within a gene's mRNA may reduce the absolute translation rate of that mRNA, especially when the relative abundance of the charged tRNA corresponding to the minor codon is low. An extension of this is that the diminution of translation rate by individual minor codons would be at least additive for multiple minor codons. Therefore, mRNAs having high relative contents of minor codons would have correspondingly low translation rates. This rate would be reflected by the synthesis of low levels of the encoded protein.

In order to reengineer the bacterial gene(s), the codon bias of the plant is determined. The codon bias is the statistical codon distribution that the plant uses for coding its proteins. After determining the bias, the percent frequency of the codons in the gene(s) of interest is determined. The primary codons preferred by the plant should be determined as well as the second and third choice of preferred codons. The amino acid sequence of the protein of interest is reverse translated so that the resulting nucleic acid sequence codes for the same protein as the native bacterial gene, but the resulting nucleic acid sequence corresponds to the first preferred codons of the desired plant. The new sequence is analyzed for restriction enzyme sites that might have been created by the modification. The identified sites are further modified by replacing the codons with second or third choice preferred codons. Other sites in the sequence which could affect the transcription or translation of the gene of interest are the exon:intron 5' or 3' junctions, poly A addition signals, or RNA polymerase termination signals. The sequence is

further analyzed and modified to reduce the frequency of TA or GC doublets. In addition to the doublets, G or C sequence blocks that have more than about four residues that are the same can affect transcription of the sequence. Therefore, these blocks are also modified by replacing the codons of first or second choice, etc. with the next preferred codon of choice. It is preferred that the plant optimized gene(s) contains about 63% of first choice codons, between about 22% to about 37% second choice codons, and between 15% and 0% third choice codons, wherein the total percentage is 100%. Most preferred the plant optimized gene(s) contain about 63% of first choice codons, at least about 22% second choice codons, about 7.5% third choice codons, and about 7.5% fourth choice codons, wherein the total percentage is 100%. The method described above enables one skilled in the art to modify gene(s) that are foreign to a particular plant so that the genes are optimally expressed in plants. The method is further illustrated in pending provisional application U.S. 60/005,405 filed on October 13, 1995, which is incorporated herein by reference.

Thus, in order to design plant optimized gene(s) the amino acid sequence of the toxins are reverse translated into a DNA sequence, utilizing a nonredundant genetic code established from a codon bias table compiled for the gene DNA sequence for the particular plant being transformed. The resulting DNA sequence, which is completely homogeneous in codon usage, is further modified to establish a DNA sequence that, besides having a higher degree of codon diversity, also contains strategically placed restriction enzyme recognition sites, desirable base composition, and a lack of sequences that might interfere with transcription of the gene, or translation of the product mRNA.

It is theorized that bacterial genes may be more easily expressed in plants if the bacterial genes are expressed in the plastids. Thus, it may be possible to express bacterial genes in plants, without optimizing the genes for plant expression, and obtain high express of the protein. See U.S. Patent Nos. 4,762,785; 5,451,513 and 5,545,817, which are incorporated herein by reference.

One of the issues regarding commercial exploiting transgenic plants is resistance management. This is of particular concern with *Bacillus thuringiensis* toxins. There are numerous companies commercially exploiting *Bacillus thuringiensis* and there has been  
5 much concern about *Bt* toxins becoming resistant. One strategy for insect resistant management would be to combine the toxins produced by *Photobhabdus* with toxins such as *Bt*, vegetative insect proteins (Ciba Geigy) or other toxins. The combinations could be formulated for a sprayable application or could be  
10 molecular combinations. Plants could be transformed with *Photobhabdus* genes that produce insect toxins and other insect toxin genes such as *Bt* as with other insect toxin genes such as *Bt*.

European Patent Application 0400246A1 describes  
15 transformation of 2 *Bt* in a plant, which could be any 2 genes. Another way to produce a transgenic plant that contains more than one insect resistant gene would be to produce two plants, with each plant containing an insect resistant gene. These plants would be backcrossed using traditional plant breeding techniques  
20 to produce a plant containing more than one insect resistant gene.

In addition to producing a transformed plant containing plant optimized gene(s), there are other delivery systems where it may be desirable to reengineer the bacterial gene(s). Along  
25 the same lines, a genetically engineered, easily isolated protein toxin fusing together both a molecule attractive to insects as a food source and the insecticidal activity of the toxin may be engineered and expressed in bacteria or in eukaryotic cells using standard, well-known techniques. After purification in the  
30 laboratory such a toxic agent with "built-in" bait could be packaged inside standard insect trap housings.

Another delivery scheme is the incorporation of the genetic material of toxins into a baculovirus vector. Baculoviruses infect particular insect hosts, including those desirably  
35 targeted with the *Photobhabdus* toxins. Infectious baculovirus harboring an expression construct for the *Photobhabdus* toxins could be introduced into areas of insect infestation to thereby intoxicate or poison infected insects.



Transfer of the insecticidal properties requires nucleic acid sequences encoding the coding the amino acid sequences for the *Phototrhabdus* toxins integrated into a protein expression vector appropriate to the host in which the vector will reside.

5 One way to obtain a nucleic acid sequence encoding a protein with insecticidal properties is to isolate the native genetic material which produces the toxins from *Phototrhabdus*, using information deduced from the toxin's amino acid sequence, large portions of which are set forth below. As described below, methods of  
10 purifying the proteins responsible for toxin activity are also disclosed.

Using N-terminal amino acid sequence data, such as set forth below, one can construct oligonucleotides complementary to all, or a section of, the DNA bases that encode the first amino acids  
15 of the toxin. These oligonucleotides can be radiolabeled and used as molecular probes to isolate the genetic material from a genomic genetic library built from genetic material isolated from strains of *Phototrhabdus*. The genetic library can be cloned in plasmid, cosmid, phage or phagemid vectors. The library could be  
20 transformed into *Escherichia coli* and screened for toxin production by the transformed cells using antibodies raised against the toxin or direct assays for insect toxicity.

This approach requires the production of a battery of oligonucleotides, since the degenerate genetic code allows an  
25 amino acid to be encoded in the DNA by any of several three-nucleotide combinations. For example, the amino acid arginine can be encoded by nucleic acid triplets CGA, CGC, CGG, CGT, AGA, and AGG. Since one cannot predict which triplet is used at those positions in the toxin gene, one must prepare oligonucleotides  
30 with each potential triplet represented. More than one DNA molecule corresponding to a protein subunit may be necessary to construct a sufficient number of oligonucleotide probes to recover all of the protein subunits necessary to achieve oral toxicity.

35 From the amino acid sequence of the purified protein, genetic materials responsible for the production of toxins can readily be isolated and cloned, in whole or in part, into an expression vector using any of several techniques well-known to one skilled in the art of molecular biology. A typical  
40 expression vector is a DNA plasmid, though other transfer means

including, but not limited to, cosmids, phagemids and phage are also envisioned. In addition to features required or desired for plasmid replication, such as an origin of replication and antibiotic resistance or other form of a selectable marker such as the *bar* gene of *Streptomyces hygroscopicus* or *viridochromogenes*, protein expression vectors normally additionally require an expression cassette which incorporates the cis-acting sequences necessary for transcription and translation of the gene of interest. The cis-acting sequences required for expression in prokaryotes differ from those required in eukaryotes and plants.

A eukaryotic expression cassette requires a transcriptional promoter upstream (5') to the gene of interest, a transcriptional termination region such as a poly-A addition site, and a ribosome binding site upstream of the gene of interest's first codon. In bacterial cells, a useful transcriptional promoter that could be included in the vector is the T7 RNA Polymerase-binding promoter. Promoters, as previously described herein, are known to efficiently promote transcription of mRNA. Also upstream from the gene of interest the vector may include a nucleotide sequence encoding a signal sequence known to direct a covalently linked protein to a particular compartment of the host cells such as the cell surface.

Insect viruses, or baculoviruses, are known to infect and adversely affect certain insects. The affect of the viruses on insects is slow, and viruses do not stop the feeding of insects. Thus viruses are not viewed as being useful as insect pest control agents. Combining the *Photographus* toxins genes into a baculovirus vector could provide an efficient way of transmitting the toxins while increasing the lethality of the virus. In addition, since different baculoviruses are specific to different insects, it may be possible to use a particular toxin to selectively target particularly damaging insect pests. A particularly useful vector for the toxins genes is the nuclear polyhedrosis virus. Transfer vectors using this virus have been described and are now the vectors of choice for transferring foreign genes into insects. The virus-toxin gene recombinant may be constructed in an orally transmissible form. Baculoviruses normally infect insect victims through the mid-gut intestinal mucosa. The toxin gene inserted behind a strong viral coat

protein promoter would be expressed and should rapidly kill the infected insect.

In addition to an insect virus or baculovirus or transgenic plant delivery system for the protein toxins of the present invention, the proteins may be encapsulated using *Bacillus thuringiensis* encapsulation technology such as but not limited to U.S. Patent Nos. 4,695,455; 4,695,462; 4,861,595 which are all incorporated herein by reference. Another delivery system for the protein toxins of the present invention is formulation of the protein into a bait matrix, which could then be used in above and below ground insect bait stations. Examples of such technology include but are not limited to PCT Patent Application WO 93/23998, which is incorporated herein by reference.

As is described above, it might become necessary to modify the sequence encoding the protein when expressing it in a non-native host, since the codon preferences of other hosts may differ from that of *Photorhabdus*. In such a case, translation may be quite inefficient in a new host unless compensating modifications to the coding sequence are made. Additionally, modifications to the amino acid sequence might be desirable to avoid inhibitory cross-reactivity with proteins of the new host, or to refine the insecticidal properties of the protein in the new host. A genetically modified toxin gene might encode a toxin exhibiting, for example, enhanced or reduced toxicity, altered insect resistance development, altered stability, or modified target species specificity.

In addition to the *Photorhabdus* genes encoding the toxins, the scope of the present invention is intended to include related nucleic acid sequences which encode amino acid biopolymers homologous to the toxin proteins and which retain the toxic effect of the *Photorhabdus* proteins in insect species after oral ingestion.

For instance, the toxins used in the present invention seem to first inhibit larval feeding before death ensues. By manipulating the nucleic acid sequence of *Photorhabdus* toxins or its controlling sequences, genetic engineers placing the toxin gene into plants could modulate its potency or its mode of action to, for example, keep the eating-inhibitory activity while eliminating the absolute toxicity to the larvae. This change could permit the transformed plant to survive until harvest

without having the unnecessarily dramatic effect on the ecosystem of wiping out all target insects. All such modifications of the gene encoding the toxin, or of the protein encoded by the gene, are envisioned to fall within the scope of the present invention.

5 Other envisioned modifications of the nucleic acid include the addition of targeting sequences to direct the toxin to particular parts of the insect larvae for improving its efficiency.

10 Strains ATCC 55397, 43948, 43949, 43950, 43951, 43952 have been deposited in the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852 USA. Amino acid and nucleotide sequence data for the W-14 native toxin (ATCC 55397) is presented below. Isolation of the genomic DNA for the toxins from the bacterial hosts is also exemplified herein.

15 Standard and molecular biology techniques were followed and taught in the specification herein. Additional information may be found in Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989), Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, which is incorporated herein by reference.

20

The following abbreviations are used throughout the Examples: Tris = tris (hydroxymethyl) amino methane; SDS = sodium dodecyl sulfate; EDTA = ethylenediaminetetraacetic acid, IPTG = isopropylthio-B-galactoside, X-gal = 5-bromo-4-chloro-3-indoyl-B-  
25 D-galactoside, CTAB = cetyltrimethylammonium bromide; kbp = kilobase pairs; dATP, dCTP, dGTP, dTTP, I = 2'-deoxynucleoside 5'-triphosphates of adenine, cytosine, guanine, thymine, and inosine, respectively; ATP = adenosine 5' triphosphate.

30

#### Example 1

#### Purification of toxin from *P. luminescens* and Demonstration of toxicity after oral delivery of purified toxin

35 The insecticidal protein toxin of the present invention was purified from *P. luminescens* strain W-14, ATCC Accession Number 55397. Stock cultures of *P. luminescens* were maintained on petri dishes containing 2% Proteose Peptone No. 3 (i.e., PP3, Difco Laboratories, Detroit MI) in 1.5% agar, incubated at 25°C and transferred weekly. Colonies of the primary form of the bacteria  
40 were inoculated into 200 ml of PP3 broth supplemented with 0.5%

polyoxyethylene sorbitan mono-stearate (Tween 60, Sigma Chemical Company, St. Louis MO) in a one liter flask. The broth cultures were grown for 72 hours at 30°C on a rotary shaker. The toxin proteins can be recovered from cultures grown in the presence or  
5 absence of Tween; however, the absence of Tween can affect the form of the bacteria grown and the profile of proteins produced by the bacteria. In the absence of Tween, a variant shift occurs insofar as the molecular weight of at least one identified toxin subunit shifts from about 200 kDa to about 185 kDa.

10 The 72 hour cultures were centrifuged at 10,000 x g for 30 minutes to remove cells and debris. The supernatant fraction that contained the insecticidal activity was decanted and brought to 50 mM K<sub>2</sub>HPO<sub>4</sub> by adding an appropriate volume of 1.0 M K<sub>2</sub>HPO<sub>4</sub>. The pH was adjusted to 8.6 by adding potassium hydroxide. This  
15 supernatant fraction was then mixed with DEAE-Sepharcel (Pharmacia LKB Biotechnology) which had been equilibrated with 50 mM K<sub>2</sub>HPO<sub>4</sub>. The toxic activity was adsorbed to the DEAE resin. This mixture was then poured into a 2.6 x 40 cm column and washed with 50 mM K<sub>2</sub>HPO<sub>4</sub> at room temperature at a flow rate of 30 ml/hr until the  
20 effluent reached a steady baseline UV absorbance at 280 nm. The column was then washed with 150 mM KCl until the effluent again reached a steady 280 nm baseline. Finally the column was washed with 300 mM KCl and fractions were collected.

Fractions containing the toxin were pooled and filter  
25 sterilized using a 0.2 micron pore membrane filter. The toxin was then concentrated and equilibrated to 100 mM KPO<sub>4</sub>, pH 6.9, using an ultrafiltration membrane with a molecular weight cutoff of 100 kDa at 4°C (Centriprep 100, Amicon Division-W.R. Grace and Company). A 3 ml sample of the toxin concentrate was applied to  
30 the top of a 2.6 x 95 cm Sephacryl S-400 HR gel filtration column (Pharmacia LKB Biotechnology). The eluent buffer was 100 mM KPO<sub>4</sub>, pH 6.9, which was run at a flow rate of 17 ml/hr, at 4°C. The effluent was monitored at 280 nm.

Fractions were collected and tested for toxic activity.  
35 Toxicity of chromatographic fractions was examined in a biological assay using *Manduca sexta* larvae. Fractions were either applied directly onto the insect diet (Gypsy moth wheat germ diet, ICN Biochemicals Division - ICN Biomedicals, Inc.) or administered by intrahemocelic injection of a 5 ul sample through  
40 the first proleg of 4th or 5th instar larva using a 30 gauge

needle. The weight of each larva within a treatment group was recorded at 24 hour intervals. Toxicity was presumed if the insect ceased feeding and died within several days of consuming treated insect diet or if death occurred within 24 hours after  
5 injection of a fraction.

The toxic fractions were pooled and concentrated using the Centriprep-100 and were then analyzed by HPLC using a 7.5 mm x 60 cm TSK-GEL G-4000 SW gel permeation column with 100 mM potassium phosphate, pH 6.9 eluent buffer running at 0.4 ml/min. This  
10 analysis revealed the toxin protein to be contained within a single sharp peak that eluted from the column with a retention time of approximately 33.6 minutes. This retention time corresponded to an estimated molecular weight of 1,000 kDa. Peak fractions were collected for further purification while fractions  
15 not containing this protein were discarded. The peak eluted from the HPLC absorbs UV light at 218 and 280 nm but did not absorb at 405 nm. Absorbance at 405 nm was shown to be an attribute of xenorhabdin antibiotic compounds.

Electrophoresis of the pooled peak fractions in a non-denaturing agarose gel (Metaphor Agarose, FMC BioProducts) showed  
20 that two protein complexes are present in the peak. The peak material, buffered in 50 mM Tris-HCl, pH 7.0, was separated on a 1.5% agarose stacking gel buffered with 100 mM Tris-HCl at pH 7.0 and 1.9% agarose resolving gel buffered with 200 mM Tris-borate  
25 at pH 8.3 under standard buffer conditions (anode buffer 1M Tris-HCl, pH 8.3; cathode buffer 0.025 M Tris, 0.192 M glycine). The gels were run at 13 mA constant current at 15°C until the phenol red tracking dye reached the end of the gel. Two protein bands were visualized in the agarose gels using Coomassie brilliant  
30 blue staining.

The slower migrating band was referred to as "protein band 1" and faster migrating band was referred to as "protein band 2." The two protein bands were present in approximately equal amounts. The Coomassie stained agarose gels were used as a guide  
35 to precisely excise the two protein bands from unstained portions of the gels. The excised pieces containing the protein bands were macerated and a small amount of sterile water was added. As a control, a portion of the gel that contained no protein was also excised and treated in the same manner as the gel pieces  
40 containing the protein. Protein was recovered from the gel

pieces by electroelution into 100 mM Tris-borate pH 8.3, at 100 volts (constant voltage) for two hours. Alternatively, protein was passively eluted from the gel pieces by adding an equal volume of 50 mM Tris-HCl, pH 7.0, to the gel pieces, then  
5 incubating at 30°C for 16 hours. This allowed the protein to diffuse from the gel into the buffer, which was then collected.

Results of insect toxicity tests using HPLC-purified toxin (33.6 min. peak) and agarose gel purified toxin demonstrated toxicity of the extracts. Injection of 1.5 µg of the HPLC  
10 purified protein kills within 24 hours. Both protein bands 1 and 2, recovered from agarose gels by passive elution or electroelution, were lethal upon injection. The protein concentration estimated for these samples was less than 50 ng/larva. A comparison of the weight gain and the mortality  
15 between the groups of larvae injected with protein bands 1 or 2 indicate that protein band 1 was more toxic by injection delivery.

When HPLC-purified toxin was applied to larval diet at a concentration of 7.5 µg/larva, it caused a halt in larval weight  
20 gain (24 larvae tested). The larvae begin to feed, but after consuming only a very small portion of the toxin treated diet they began to show pathological symptoms induced by the toxin and the larvae cease feeding. The insect frass became discolored and most larva showed signs of diarrhea. Significant insect  
25 mortality resulted when several 5 µg toxin doses were applied to the diet over a 7-10 day period.

Agarose-separated protein band 1 significantly inhibited larval weight gain at a dose of 200 ng/larva. Larvae fed similar concentrations of protein band 2 were not inhibited and gained  
30 weight at the same rate as the control larvae. Twelve larvae were fed eluted protein and 45 larvae were fed protein-containing agarose pieces. These two sets of data indicate that protein band 1 was orally toxic to *Manduca sexta*. In this experiment it appeared that protein band 2 was not toxic to *Manduca sexta*.

35 Further analysis of protein bands 1 and 2 by SDS-PAGE under denaturing conditions showed that each band was composed of several smaller protein subunits. Proteins were visualized by Coomassie brilliant blue staining followed by silver staining to achieve maximum sensitivity.

The protein subunits in the two bands were very similar. Protein band 1 contains 8 protein subunits of 25.1, 56.2, 60.8, 65.6, 166, 171, 184 and 208 kDa. Protein band 2 had an identical profile except that the 25.1, 60.8, and 65.6 kDa proteins were not present. The 56.2, 60.8, 65.6, and 184 kDa proteins were present in the complex of protein band 1 at approximately equal concentrations and represent 80% or more of the total protein content of that complex.

The native HPLC-purified toxin was further characterized as follows. The toxin was heat labile in that after being heated to 60°C for 15 minutes it lost its ability to kill or to inhibit weight gain when injected or fed to *M. sexta* larvae. Assays were designed to detect lipase, type C phospholipase, nuclease or red blood cell hemolysis activities and were performed with purified toxin. None of these activities were present. Antibiotic zone inhibition assays were also done and the purified toxin failed to inhibit growth of Gram-negative or -positive bacteria, yeast or filamentous fungi, indicating that the toxic is not a xenorhabdin antibiotic.

The native HPLC-purified toxin was tested for ability to kill insects other than *Manduca sexta*. Table 2 lists insects killed by the HPLC-purified *P. luminescens* toxin in this study.

Table 2  
Insects Killed by *P. luminescens* Toxin

	<u>Common Name</u>	<u>Order</u>	<u>Genus and species</u>	<u>Route of Delivery</u>
30	Tobacco horn worm	Lepidoptera	<i>Manduca sexta</i>	Oral and injected
	Mealworm	Coleoptera	<i>Tenebrio molitor</i>	Oral
35	Pharaoh ant	Hymenoptera	<i>Monomorium pharoanis</i>	Oral
	German cockroach	Dictyoptera	<i>Blattella germanica</i>	Oral and injected
40	Mosquito	Diptera	<i>Aedes aegypti</i>	Oral



Example 2  
Insecticide Utility

The *Photorhabdus luminescens* utility and toxicity were further characterized. *Photorhabdus luminescens* (strain W-14) culture broth was produced as follows. The production medium was 2% Bacto Proteose Peptone<sup>®</sup> Number 3 (PP3, Difco Laboratories, Detroit, Michigan) in Milli-Q<sup>®</sup> deionized water. Seed culture flasks consisted of 175 ml medium placed in a 500 ml tribaffled flask with a Delong neck, covered with a Kaput and autoclaved for 20 minutes, T=250°F. Production flasks consisted of 500 mls in a 2.8 liter 500 ml tribaffled flask with a Delong neck, covered by a Shin-etsu silicon foam closure. These were autoclaved for 45 minutes, T=250°F. The seed culture was incubated at 28°C at 150 rpm in a gyrotory shaking incubator with a 2 inch throw. After 16 hours of growth, 1% of the seed culture was placed in the production flask which was allowed to grow for 24 hours before harvest. Production of the toxin appears to be during log phase growth. The microbial broth was transferred to a 1L centrifuge bottle and the cellular biomass was pelleted (30 minutes at 2500 RPM at 4°C, [R.C.F. = ~1600] HG-4L Rotor RC3 Sorval centrifuge, Dupont, Wilmington, Delaware). The primary broth was chilled at 4°C for 8 - 16 hours and recentrifuged at least 2 hours (conditions above) to further clarify the broth by removal of a putative mucopolysaccharide which precipitated upon standing. (An alternative processing method combined both steps and involved the use of a 16 hour clarification centrifugation, same conditions as above.) This broth was then stored at 4°C prior to bioassay or filtration.

*Photorhabdus* culture broth and protein toxin(s) purified from this broth showed activity (mortality and/or growth inhibition, reduced adult emergence) against a number of insects. More specifically, the activity is seen against corn rootworm (larvae and adult), Colorado potato beetle, and turf grubs, which are members of the insect order Coleoptera. Other members of the Coleoptera include wireworms, pollen beetles, flea beetles, seed beetles and weevils. Activity has also been observed against aster leafhopper, which is a member of the order, Homoptera. Other members of the Homoptera include planthoppers, pear psylla, apple sucker, scale insects, whiteflies, and spittle bugs, as

- well as numerous host specific aphid species. The broth and purified fractions are also active against beet armyworm, cabbage looper, black cutworm, tobacco budworm, European corn borer, corn earworm, and codling moth, which are members of the order
- 5 *Lepidoptera*. Other typical members of this order are clothes moth, Indian mealmoth, leaf rollers, cabbage worm, cotton bollworm, bagworm, Eastern tent caterpillar, sod webworm, and fall armyworm. Activity is also seen against fruitfly and mosquito larvae, which are members of the order *Diptera*. Other
- 10 members of the order *Diptera* are pea midge, carrot fly, cabbage root fly, turnip root fly, onion fly, crane fly, house fly, and various mosquito species. Activity is seen against carpenter ant and Argentine ant, which are members of the order that also includes fire ants, odorous house ants, and little black ants.
- 15 The broth/fraction is useful for reducing populations of insects and were used in a method of inhibiting an insect population. The method may comprise applying to a locus of the insect an effective insect inactivating amount of the active described. Results are reported in Table 3.
- 20 Activity against corn rootworm larvae was tested as follows. *Photorhabdus* culture broth (filter sterilized, cell-free) or purified HPLC fractions were applied directly to the surface (~1.5 cm<sup>2</sup>) of 0.25 ml of artificial diet in 30  $\mu$ l aliquots following dilution in control medium or 10 mM sodium phosphate
- 25 buffer, pH 7.0, respectively. The diet plates were allowed to air-dry in a sterile flow-hood and the wells were infested with single, neonate *Diabrotica undecimpunctata howardi* (Southern corn rootworm, SCR) hatched from sterilized eggs, with second instar SCR grown on artificial diet or with second instar *Diabrotica*
- 30 *virgifera virgifera* (Western corn rootworm, WCR) reared on corn seedlings grown in Metromix<sup>®</sup>. Second instar larvae were weighed prior to addition to the diet. The plates were sealed, placed in a humidified growth chamber and maintained at 27°C for the appropriate period (4 days for neonate and adult SCR, 2-5 days
- 35 for WCR larvae, 7-14 days for second instar SCR). Mortality and weight determinations were scored as indicated. Generally, 16 insects per treatment were used in all studies. Control mortalities were as follows: neonate larvae, <5%, adult beetles, 5%.

Activity against Colorado potato beetle was tested as follows. *Photorhabdus* culture broth or control medium was applied to the surface (~2.0 cm<sup>2</sup>) of 1.5 ml of standard artificial diet held in the wells of a 24-well tissue culture plate. Each well

5 received 50 µl of treatment and was allowed to air dry.

Individual second instar Colorado potato beetle (*Leptinotarsa decemlineata*, CPB) larvae were then placed onto the diet and mortality was scored after 4 days. Ten larvae per treatment were used in all studies. Control mortality was 3.3%.

10 Activity against Japanese beetle grubs and beetles was tested as follows. Turf grubs (*Popillia japonica*, 2-3rd instar) were collected from infested lawns and maintained in the laboratory in soil/peat mixture with carrot slices added as additional diet. Turf beetles were pheromone-trapped locally and

15 maintained in the laboratory in plastic containers with maple leaves as food. Following application of undiluted *Photorhabdus* culture broth or control medium to corn rootworm artificial diet (30 µl/1.54 cm<sup>2</sup>, beetles) or carrot slices (larvae), both stages were placed singly in a diet well and observed for any mortality

20 and feeding. In both cases there was a clear reduction in the amount of feeding (and feces production) observed.

Activity against mosquito larvae was tested as follows. The assay was conducted in a 96-well microtiter plate. Each well contained 200 µl of aqueous solution (*Photorhabdus* culture broth,

25 control medium or H<sub>2</sub>O) and approximately 20, 1-day old larvae (*Aedes aegypti*). There were 6 wells per treatment. The results were read at 2 hours after infestation and did not change over the three day observation period. No control mortality was seen.

Activity against fruitflies was tested as follows.

30 Purchased *Drosophila melanogaster* medium was prepared using 50% dry medium and a 50% liquid of either water, control medium or *Photorhabdus* culture broth. This was accomplished by placing 8.0 ml of dry medium in each of 3 rearing vials per treatment and adding 8.0 ml of the appropriate liquid. Ten late instar

35 *Drosophila melanogaster* maggots were then added to each vial. The vials were held on a laboratory bench, at room temperature, under fluorescent ceiling lights. Pupal or adult counts were made after 3, 7 and 10 days of exposure. Incorporation of *Photorhabdus* culture broth into the diet media for fruitfly

maggots caused a slight (17%) but significant reduction in day-10 adult emergence as compared to water and control medium (3% reduction).

Activity against aster leafhopper was tested as follows.

- 5 The ingestion assay for aster leafhopper (*Macrosteles severini*) is designed to allow ingestion of the active without other external contact. The reservoir for the active/"food" solution is made by making 2 holes in the center of the bottom portion of a 35 x 10 mm Petri dish. A 2 inch Parafilm M<sup>®</sup> square is placed  
10 across the top of the dish and secured with an "O" ring. A 1 oz. plastic cup is then infested with approximately 7 leafhoppers and the reservoir is placed on top of the cup, Parafilm down. The test solution is then added to the reservoir through the holes. In tests using undiluted *Photorhabdus* culture broth, the broth  
15 and control medium were dialyzed against water to reduce control mortality. Mortality is reported at day 2 where 26.5% control mortality was seen. In the tests using purified fractions (200 mg protein/ml ) a final concentration of 5% sucrose was used in all treatments to improve survivability of the aster leafhoppers.  
20 The assay was held in an incubator at 28°C, 70% RH with a 16/8 photoperiod. The assay was graded for mortality at 72 hours. Control mortality was 5.5%.

- Activity against Argentine ants was tested as follows. A 1.5 ml aliquot of 100% *Photorhabdus* culture broth, control medium  
25 or water was pipetted into 2.0 ml clear glass vials. The vials were plugged with a piece of cotton dental wick that was moistened with the appropriate treatment. Each vial was placed into a separate 60x16mm Petri dish with 8 to 12 adult Argentine ants (*Linepithema humile*). There were three replicates per  
30 treatment. Bioassay plates were held on a laboratory bench, at room temperature under fluorescent ceiling lights. Mortality readings were made after 5 days of exposure. Control mortality was 24%.

- Activity against carpenter ant was tested as follows. Black  
35 carpenter ant workers (*Camponotus pennsylvanicus*) were collected from trees on DowElanco property in Indianapolis, IN. Tests with *Photorhabdus* culture broth were performed as follows. Each plastic bioassay container (7 1/8" x 3") held fifteen workers, a paper harborage and 10 ml of broth or control media in a plastic  
40 shot glass. A cotton wick delivered the treatment to the ants

through a hole in the shot glass lid. All treatments contained 5% sucrose. Bioassays were held in the dark at room temperature and graded at 19 days. Control mortality was 9%. Assays delivering purified fractions utilized artificial ant diet mixed with the treatment (purified fraction or control solution) at a rate of 0.2 ml treatment/2.0 g diet in a plastic test tube. The final protein concentration of the purified fraction was less than 10 µg/g diet. Ten ants per treatment, a water source, harborage and the treated diet were placed in sealed plastic containers and maintained in the dark at 27°C in a humidified incubator. Mortality was scored at day 10. No control mortality was seen.

Activity against various lepidopteran larvae was tested as follows. *Photographus* culture broth or purified fractions were applied directly to the surface (~1.5 cm<sup>2</sup>) of 0.25 ml of standard artificial diet in 30 µl aliquots following dilution in control medium or 10 mM sodium phosphate buffer, pH 7.0, respectively. The diet plates were allowed to air-dry in a sterile flow-hood and the wells were infested with single, neonate larva. European corn borer (*Ostrinia nubilalis*) and corn earworm (*Helioverpa zea*) eggs were supplied from commercial sources and hatched in-house, whereas beet armyworm (*Spodoptera exigua*), cabbage looper (*Trichoplusia ni*), tobacco budworm (*Heliothis virescens*), codling moth (*Laspeyresia pomonella*) and black cutworm (*Agrotis ipsilon*) larvae were supplied internally. Following infestation with larvae, the diet plates were sealed, placed in a humidified growth chamber and maintained in the dark at 27°C for the appropriate period. Mortality and weight determinations were scored at days 5-7 for *Photographus* culture broth and days 4-7 for the purified fraction. Generally, 16 insects per treatment were used in all studies. Control mortality ranged from 4-12.5% for control medium and was less than 10% for phosphate buffer.

Table 3

Effect of *Photographus luminescens* (strain W-14)  
Culture Broth and Purified Toxin Fraction on Mortality and Growth  
Inhibition of Different Insect Orders/Species

5

Insect Order/Species	Broth		Purified Fraction	
	% Mort.	% G.I.	% Mort.	% G.I.
<b>COLEOPTERA</b>				
Corn Rootworm				
Southern/neonate larva	100	na	100	na
Southern/2 <sup>nd</sup> instar	na	38.5	nt	nt
Southern/adult	45	nt	nt	nt
Western/2 <sup>nd</sup> instar	na	35	nt	nt
Colorado Potato				
Beetle	93	nt	nt	nt
2 <sup>nd</sup> instar				
Turf Grub	na	a.f.	nt	nt
3 <sup>rd</sup> instar	na	a.f.	nt	nt
adult				
<b>DIPTERA</b>				
Fruit Fly (adult	17	nt	nt	nt
emergence)	100	na	nt	nt
Mosquito larvae				
<b>HOMOPTERA</b>				
Aster Leafhopper	96.5	na	100	na
<b>HYMENOPTERA</b>				
Argentine Ant	75	na	nt	na
Carpenter Ant	71	na	100	na
<b>LEPIDOPTERA</b>				
Beet Armyworm	12.5	36	18.75	41.4
Black Cutworm	nt	nt	0	71.2
Cabbage Looper	nt	nt	21.9	66.8
Codling Moth	nt	nt	6.25	45.9
Corn Earworm	56.3	94.2	97.9	na
European Corn Borer	96.7	98.4	100	na
Tobacco Budworm	13.5	52.5	19.4	85.6

Mort. = mortality, G.I. = growth inhibition,  
na = not applicable, nt = not tested, a.f. = anti-feedant

Example 3Insecticide Utility Upon Soil Application

*Photorhabdus luminescens* (strain W-14) culture broth was shown to be active against corn rootworm when applied directly to soil or a soil-mix (Metromix®). Activity against neonate SCR and WCR in Metromix® was tested as follows (Table 4). The test was run using corn seedlings (United Agriseeds brand CL614) that were germinated in the light on moist filter paper for 6 days. After roots were approximately 3-6 cm long, a single kernel/seedling was planted in a 591 ml clear plastic cup with 50 gm of dry Metromix®. Twenty neonate SCR or WCR were then placed directly on the roots of the seedling and covered with Metromix®. Upon infestation, the seedlings were then drenched with 50 ml total volume of a diluted broth solution. After drenching, the cups were sealed and left at room temperature in the light for 7 days. Afterwards, the seedlings were washed to remove all Metromix® and the roots were excised and weighed. Activity was rated as the percentage of corn root remaining relative to the control plants and as leaf damage induced by feeding. Leaf damage was scored visually and rated as either -, +, ++, or +++, with - representing no damage and +++ representing severe damage.

Activity against neonate SCR in soil was tested as follows (Table 5). The test was run using corn seedlings (United Agriseeds brand CL614) that were germinated in the light on moist filter paper for 6 days. After the roots were approximately 3-6 cm long, a single kernel/seedling was planted in a 591 ml clear plastic cup with 150 gm of soil from a field in Lebanon, IN planted the previous year with corn. This soil had not been previously treated with insecticides. Twenty neonate SCR were then placed directly on the roots of the seedling and covered with soil. After infestation, the seedlings were drenched with 50 ml total volume of a diluted broth solution. After drenching, the unsealed cups were incubated in a high relative humidity chamber (80%) at 78°F. Afterwards, the seedlings were washed to remove all soil and the roots were excised and weighed. Activity was rated as the percentage of corn root remaining relative to the control plants and as leaf damage induced by feeding. Leaf damage was scored visually and rated as either -, +, ++, or +++, with - representing no damage and +++ representing severe damage.

Table 4  
Effect of *Photorhabdus luminescens* (strain W-14) Culture Broth on Rootworm Larvae After Post-Infestation Drenching (Metromix®)

5

	Treatment	Larvae	Leaf Damage	Root Weight (g)	%
	<b>Southern Corn Rootworm</b>				
10	Water	-	-	0.4916 ± 0.023	100
	Medium (2.0% v/v)	-	-	0.4416 ± 0.029	100
	Broth (6.25% v/v)	-	-	0.4641 ± 0.081	100
	Water	+	+++	0.1410 ± 0.006	28.7
15	Media (2.0% v/v)	+	+++	0.1345 ± 0.028	30.4
	Broth (1.56% v/v)	+	-	0.4830 ± 0.031	104
	<b>Western Corn Rootworm</b>				
20	Water	-	-	0.4446 ± 0.019	100
	Broth (2.0% v/v)	-	-	0.4069 ± 0.026	100
	Water	+	-	0.2202 ± 0.015	49
25	Broth (2.0% v/v)	+	-	0.3879 ± 0.013	95

Table 5  
Effect of *Photorhabdus luminescens* (strain W-14) Culture Broth on Southern Corn Rootworm Larvae After Post-Infestation Drenching (Soil)

30

	Treatment	Larvae	Leaf Damage	Root Weight (g)	%
	Water	-	-	0.2148 ± 0.014	100
35	Broth (50% v/v)	-	-	0.2260 ± 0.016	103
	Water	+	+++	0.0916 ± 0.009	43
	Broth (50% v/v)	+	-	0.2428 ± 0.032	113

40 Activity of *Photorhabdus luminescens* (strain W-14) culture broth against second instar turf grubs in Metromix® was observed in tests conducted as follows (Table 6). Approximately 50 gm of dry Metromix® was added to a 591 ml clear plastic cup. The Metromix® was then drenched with 50 ml total volume of a 50% (v/v) diluted *Photorhabdus* broth solution. The dilution of crude broth was made with water, with 50% broth being prepared by adding 25 ml of crude broth to 25 ml of water for 50 ml total volume. A 1% (w/v) solution of proteose peptone #3 (PP3), which is a 50% dilution of the normal media concentration, was used as a broth control. After drenching, five second instar turf grubs were

50



- placed on the top of the moistened Metromix<sup>®</sup>. Healthy turf grub larvae burrowed rapidly into the Metromix<sup>®</sup>. Those larvae that did not burrow within 1h were removed and replaced with fresh larvae. The cups were sealed and placed in a 28°C incubator, in the dark.
- 5 After seven days, larvae were removed from the Metromix<sup>®</sup> and scored for mortality. Activity was rated the percentage of mortality relative to control.

10 Table 6  
Effect of *Photorhabdus luminescens* (strain W-14) Culture Broth on Turf Grub After Pre-Infestation Drenching (Metromix<sup>®</sup>)

15	Treatment	Mortality*	Mortality %
	Water	7/15	47
20	Control medium (1.0% w/v)	12/19	63
	Broth (50% v/v)	17/20	85

25 \*expressed as a ratio of dead/living larvae

#### Example 4

#### Insecticide Utility Upon Leaf Application

- 30 Activity of *Photorhabdus* broth against European corn borer was seen when the broth was applied directly to the surface of maize leaves (Table 7). In these assays *Photorhabdus* broth was diluted 100-fold with culture medium and applied manually to the surface of excised maize leaves at a rate of ~6.0 µl/cm<sup>2</sup> of leaf
- 35 surface. The leaves were air dried and cut into equal sized strips approximately 2 x 2 inches. The leaves were rolled, secured with paper clips and placed in 1 oz plastic shot glasses with 0.25 inch of 2% agar on the bottom surface to provide moisture. Twelve neonate European corn borers were then placed
- 40 onto the rolled leaf and the cup was sealed. After incubation for 5 days at 27°C in the dark, the samples were scored for feeding damage and recovered larvae.

Table 7

Effect of *Photorhabdus luminescens* (strain W-14) Culture Broth on European Corn Borer Larvae Following Pre-Infestation Application to Excised Maize Leaves

Treatment	Leaf Damage	Larvae Recovered	Weight (mg)
Water	Extensive	55/120	0.42 mg
Control Medium	Extensive	40/120	0.50 mg
Broth (1.0% v/v)	Trace	3/120	0.15 mg

10

Activity of the culture broth against neonate tobacco budworm (*Heliothis virescens*) was demonstrated using a leaf dip methodology. Fresh cotton leaves were excised from the plant and leaf disks were cut with an 18.5 mm cork-borer. The disks were individually emersed in control medium (PP3) or *Photorhabdus luminescens* (strain W-14) culture broth which had been concentrated approximately 10-fold using an Amicon (Beverly, MA), Proflux M12 tangential filtration system with a 10 kDa filter. Excess liquid was removed and a straightened paper clip was placed through the center of the disk. The paper clip was then wedged into a plastic, 1.0 oz shot glass containing approximately 2.0 ml of 1% Agar. This served to suspend the leaf disk above the agar. Following drying of the leaf disk, a single neonate tobacco budworm larva was placed on the disk and the cup was capped. The cups were then sealed in a plastic bag and placed in a darkened, 27°C incubator for 5 days. At this time the remaining larvae and leaf material were weighed to establish a measure of leaf damage (Table 8).

30

Table 8

Effect of *Photorhabdus luminescens* (Strain W-14) Culture Broth on Tobacco Budworm Neonates in a Cotton-Leaf Dip Assay

Treatment	Leaf Disk	Final Weights (mg)
		Larvae
Control leaves	55.7 ± 1.3	na*
Control Medium	34.0 ± 2.9	4.3 ± 0.91
<i>Photorhabdus</i> broth	54.3 ± 1.4	0.0**

\* - not applicable, \*\* - no live larvae found

40

Example 5, Part A  
Characterization of Toxin Peptide Components

In a subsequent analysis, the toxin protein subunits of the  
5 bands isolated as in Example 1 were resolved on a 7% SDS  
polyacrylamide electrophoresis gel with a ratio of 30:0.8  
(acrylamide:BIS-acrylamide). This gel matrix facilitates better  
resolution of the larger proteins. The gel system used to  
estimate the Band 1 and Band 2 subunit molecular weights in  
10 Example 1 was an 18% gel with a ratio of 38:0.18 (acrylamide:BIS-  
acrylamide), which allowed for a broader range of size  
separation, but less resolution of higher molecular weight  
components.

In this analysis, 10, rather than 8, protein bands were  
15 resolved. Table 9 reports the calculated molecular weights of  
the 10 resolved bands, and directly compares the molecular  
weights estimated under these conditions to those of the prior  
example. It is not surprising that additional bands were  
detected under the different separation conditions used in this  
20 example. Variations between the prior and new estimates of  
molecular weight are also to be expected given the differences in  
analytical conditions. In the analysis of this example, it is  
thought that the higher molecular weight estimates are more  
accurate than in Example 1, as a result of improved resolution.  
25 However, these are estimates based on SDS PAGE analysis, which  
are typically not analytically precise and result in estimates of  
peptides and which may have been further altered due to post- and  
co-translational modifications.

Amino acid sequences were determined for the N-terminal  
30 portions of five of the 10 resolved peptides. Table 9 correlates  
the molecular weight of the proteins and the identified  
sequences. In SEQ ID NO:2, certain analyses suggest that the  
proline at residue 5 may be an asparagine (asn). In SEQ ID NO:3,  
certain analyses suggest that the amino acid residues at  
35 positions 13 and 14 are both arginine (arg). In SEQ ID NO:4,  
certain analyses suggest that the amino acid residue at position  
6 may be either alanine (ala) or serine (ser). In SEQ ID NO:5,  
certain analyses suggest that the amino acid residue at position  
3 may be aspartic acid (asp).

40

Table 3

	EXAMPLE 1		
	<u>ESTIMATE</u>	<u>NEW ESTIMATE*</u>	<u>SEQ. LISTING</u>
	208	200.2 kDa	SEQ ID NO:1
5	184	175.0 kDa	SEQ ID NO:2
	65.6	68.1 kDa	SEQ ID NO:3
	60.8	65.1 kDa	SEQ ID NO:4
	56.2	58.3 kDa	SEQ ID NO:5
	25.1	23.2 kDa	SEQ ID NO:15
10	*New estimates are based on SDS PAGE and are not based on gene sequences. SDS PAGE is not analytically precise.		

Example 5, Part BCharacterization of Toxin Peptide Components

15

New N-terminal sequence, SEQ ID NO:15, Ala Gln Asp Gly Asn Gln Asp Thr Phe Phe Ser Gly Asn Thr, was obtained by further N-terminal sequencing of peptides isolated from Native HPLC-purified toxin as described in Example 5, Part A, above. This peptide comes from the *tcaA* gene. The peptide labeled TcaAii, starts at position 254 and goes to position 491, where the TcaAiii peptide starts, SEQ ID NO:4. The estimated size of the peptide based on the gene sequence is 25,240 Da.

25

Example 6Characterization of Toxin Peptide Components

In yet another analysis, the toxin protein complex was re-isolated from the *Photobacterium luminescens* growth medium (after culture without Tween) by performing a 10% - 80% ammonium sulfate precipitation followed by an ion exchange chromatography step (Mono Q) and two molecular sizing chromatography steps. These conditions were like those used in Example 1. During the first molecular sizing step, a second biologically active peak was found at about  $100 \pm 10$  kDa. Based upon protein measurements, this fraction was 20 - 50 fold less active than the larger, or primary, active peak of about  $860 \pm 100$  kDa (native). During this isolation experiment, a smaller active peak of about  $325 \pm 50$  kDa that retained a considerable portion of the starting biological activity was also resolved. It is thought that the 325 kDa peak is related to or derived from the 860 kDa peak.

A 56 kDa protein was resolved in this analysis. The N-terminal sequence of this protein is presented in SEQ ID NO:6. It is noteworthy that this protein shares significant identity and conservation with SEQ ID NO:5 at the N-terminus, suggesting that the two may be encoded by separate members of a gene family and that the proteins produced by each gene are sufficiently similar to both be operable in the insecticidal toxin complex.

A second, prominent 185 kDa protein was consistently present in amounts comparable to that of protein 3 from Table 9, and may be the same protein or protein fragment. The N-terminal sequence of this 185 kDa protein is shown at SEQ ID NO:7.

Additional N-terminal amino acid sequence data were also obtained from isolated proteins. None of the determined N-terminal sequences appear identical to a protein identified in Table 9. Other proteins were present in isolated preparation. One such protein has an estimated molecular weight of 108 kDa and an N-terminal sequence as shown in SEQ ID NO:8. A second such protein has an estimated molecular weight of 80 kDa and an N-terminal sequence as shown in SEQ ID NO:9.

When the protein material in the approximately 325 kDa active peak was analyzed by size, bands of approximately 51, 31, 28, and 22 kDa were observed. As in all cases in which a molecular weight was determined by analysis of electrophoretic mobility, these molecular weights were subject to error effects introduced by buffer ionic strength differences, electrophoresis power differences, and the like. One of ordinary skill would understand that definitive molecular weight values cannot be determined using these standard methods and that each was subject to variation. It was hypothesized that proteins of these sizes are degradation products of the larger protein species (of approximately 200 kDa size) that were observed in the larger primary toxin complex.

Finally, several preparations included a protein having the N-terminal sequence shown in SEQ ID NO:10. This sequence was strongly homologous to known chaperonin proteins, accessory proteins known to function in the assembly of large protein complexes. Although the applicants could not ascribe such an assembly function to the protein identified in SEQ ID NO:10, it was consistent with the existence of the described toxin protein complex that such a chaperonin protein could be involved in its

assembly. Moreover, although such proteins have not directly been suggested to have toxic activity, this protein may be important to determining the overall structural nature of the protein toxin, and thus, may contribute to the toxic activity or durability of the complex *in vivo* after oral delivery.

Subsequent analysis of the stability of the protein toxin complex to proteinase K was undertaken. It was determined that after 24 hour incubation of the complex in the presence of a 10-fold molar excess of proteinase K, activity was virtually eliminated (mortality on oral application dropped to about 5%). These data confirm the proteinaceous nature of the toxin.

The toxic activity was also retained by a dialysis membrane, again confirming the large size of the native toxin complex.

15

#### Example 7

##### Isolation, Characterization and Partial Amino Acid Sequencing of *Photorhabdus* Toxins

Isolation and N-Terminal Amino Acid Sequencing: In a set of experiments conducted in parallel to Examples 5 and 6, ammonium sulfate precipitation of *Photorhabdus* proteins was performed by adjusting *Photorhabdus* broth, typically 2-3 liters, to a final concentration of either 10% or 20% by the slow addition of ammonium sulfate crystals. After stirring for 1 hour at 4°C, the material was centrifuged at 12,000 x g for 30 minutes. The supernatant was adjusted to 80% ammonium sulfate, stirred at 4°C for 1 hour, and centrifuged at 12,000 x g for 60 minutes. The pellet was resuspended in one-tenth the volume of 10 mM Na<sub>2</sub>·PO<sub>4</sub>, pH 7.0 and dialyzed against the same phosphate buffer overnight at 4°C. The dialyzed material was centrifuged at 12,000 x g for 1 hour prior to ion exchange chromatography.

A HR 16/50 Q Sepharose (Pharmacia) anion exchange column was equilibrated with 10 mM Na<sub>2</sub>·PO<sub>4</sub>, pH 7.0. Centrifuged, dialyzed ammonium sulfate pellet was applied to the Q Sepharose column at a rate of 1.5 ml/min and washed extensively at 3.0 ml/min with equilibration buffer until the optical density (O.D. 280) reached less than 0.100. Next, either a 60 minute NaCl gradient ranging from 0 to 0.5 M at 3 ml/min, or a series of step elutions using 0.1 M, 0.4 M and finally 1.0 NaCl for 60 minutes each was applied to the column. Fractions were pooled and concentrated using a

Centriprep 100. Alternatively, proteins could be eluted by a single 0.4 M NaCl wash without prior elution with 0.1 M NaCl.

Two milliliter aliquots of concentrated Q Sepharose samples were loaded at 0.5 ml/min onto a HR 16/50 Superose 12 (Pharmacia) gel filtration column equilibrated with 10 mM  $\text{Na}_2\text{PO}_4$ , pH 7.0. The column was washed with the same buffer for 240 min at 0.5 ml/min and 2 min samples were collected. The void volume material was collected and concentrated using a Centriprep 100. Two milliliter aliquots of concentrated Superose 12 samples were loaded at 0.5 ml/min onto a HR 16/50 Sepharose 4B-CL (Pharmacia) gel filtration column equilibrated with 10 mM  $\text{Na}_2\text{PO}_4$ , pH 7.0. The column was washed with the same buffer for 240 min at 0.5 ml/min and 2 min samples were collected.

The excluded protein peak was subjected to a second fractionation by application to a gel filtration column that used a Sepharose CL-4B resin, which separates proteins ranging from ~30 kDa to 1000 kDa. This fraction was resolved into two peaks; a minor peak at the void volume (>1000 kDa) and a major peak which eluted at an apparent molecular weight of about 860 kDa. Over a one week period subsequent samples subjected to gel filtration showed the gradual appearance of a third peak (approximately 325 kDa) that seemed to arise from the major peak, perhaps by limited proteolysis. Bioassays performed on the three peaks showed that the void peak had no activity, while the 860 kDa toxin complex fraction was highly active, and the 325 kDa peak was less active, although quite potent. SDS PAGE analysis of Sepharose CL-4B toxin complex peaks from different fermentation productions revealed two distinct peptide patterns, denoted "P" and "S". The two patterns had marked differences in the molecular weights and concentrations of peptide components in their fractions. The "S" pattern, produced most frequently, had 4 high molecular weight peptides (> 150 kDa) while the "P" pattern had 3 high molecular weight peptides. In addition, the "S" peptide fraction was found to have 2-3 fold more activity against European Corn Borer. This shift may be related to variations in protein expression due to age of inoculum and/or other factors based on growth parameters of aged cultures.

Milligram quantities of peak toxin complex fractions determined to be "P" or "S" peptide patterns were subjected to preparative SDS PAGE, and transblotted with TRIS-glycine

(Seprabuff™ to PVDF membranes (ProBlott™, Applied Biosystems) for 3-4 hours. Blots were sent for amino acid analysis and N-terminal amino acid sequencing at Harvard MicroChem and Cambridge ProChem, respectively. Three peptides in the "S" pattern had  
5 unique N-terminal amino acid sequences compared to the sequences identified in the previous example. A 201 kDa (TcdA<sub>ii</sub>) peptide set forth as SEQ ID NO:13 below shared between 33% amino acid identity and 50% similarity with SEQ ID NO:1 (TcbA<sub>ii</sub>) (Table 10, in Table 10 vertical lines denote amino acid identities and  
10 colons indicate conservative amino acid substitutions). A second peptide of 197 kDa, SEQ ID NO:14 (TcdB), had 42% identity and 58% homology with SEQ ID NO:2 (TcaC). Yet a third peptide of 205 kDa was denoted TcdA<sub>ii</sub>. In addition, a limited N-terminal amino acid sequence, SEQ ID NO:16 (TcbA), of a peptide of at least 235 kDa  
15 was identical in homology with the amino acid sequence, SEQ ID NO:12, deduced from a cloned gene (*tcbA*), SEQ ID NO:11, containing a deduced amino acid sequence corresponding to SEQ ID NO:1 (TcbA<sub>ii</sub>). This indicates that the larger 235+ kDa peptide was proteolytically processed to the 201 kDa peptide, (TcbA<sub>ii</sub>),  
20 (SEQ ID NO:1) during fermentation, possibly resulting in activation of the molecule. In yet another sequence, the sequence originally reported as SEQ ID NO:5 (TcaB<sub>ii</sub>) reported in Example 5 above, was found to contain an aspartic acid residue (Asp) at the third position rather than glycine (Gly) and two  
25 additional amino acids Gly and Asp at the eighth and ninth positions, respectively. In yet two other sequences, SEQ ID NO:2 (TcaC) and SEQ ID NO:3 (TcaB<sub>i</sub>), additional amino acid sequence was obtained. Densitometric quantitation was performed using a sample that was identical to the "S" preparation sent for N-  
30 terminal analysis. This analysis showed that the 201 kDa and 197 kDa peptides represent 7.0% and 7.2%, respectively, of the total Coomassie brilliant blue stained protein in the "S" pattern and are present in amounts similar to the other abundant peptides. It is speculated that these peptides may represent protein  
35 homologs, analogous to the situation found with other bacterial toxins, such as various CryI Bt toxins. These proteins vary from 40-90% homology at their N-terminal amino acid sequence, which encompasses the toxic fragment.



Internal Amino Acid Sequencing: To facilitate cloning of toxin peptide genes, internal amino acid sequences of selected peptides were obtained as followed. Milligram quantities of peak 2A fractions determined to be "P" or "S" peptide patterns were subjected to preparative SDS PAGE, and transblotted with TRIS-glycine (Seprabuff™ to PVDF membranes (ProBlott™, Applied Biosystems) for 3-4 hours. Blots were sent for amino acid analysis and N-terminal amino acid sequencing at Harvard MicroChem and Cambridge ProChem, respectively. Three peptides, referred to as TcbA<sub>ii</sub> (containing SEQ ID NO:1), TcdA<sub>ii</sub>, and TcaB<sub>i</sub> (containing SEQ ID NO:3) were subjected to trypsin digestion by Harvard MicroChem followed by HPLC chromatography to separate individual peptides. N-terminal amino acid analysis was performed on selected tryptic peptide fragments. Two internal peptides were sequenced for the peptide TcaB<sub>i</sub> (205 kDa peptide) referred to as TcaB<sub>i</sub>-PT111 (SEQ ID NO:17) and TcaB<sub>i</sub>-PT79 (SEQ ID NO:18). Two internal peptides were sequenced for the peptide TcaB<sub>i</sub> (68 kDa peptide) referred to as TcaB<sub>i</sub>-PT158 (SEQ ID NO:19) and TcaB<sub>i</sub>-PT108 (SEQ ID NO:20). Four internal peptides were sequenced for the peptide TcbA<sub>ii</sub> (201 kDa peptide) referred to as TCBAII-PT103 (SEQ ID NO:21), TcbA<sub>ii</sub>-PT56 (SEQ ID NO:22), TcbA<sub>ii</sub>-PT81(a) (SEQ ID NO:23), and TcbA<sub>ii</sub>-PT81(b) (SEQ ID NO:24).

Table 10

#### N-Terminal Amino Acid Sequences

	201 kDa (33% identity & 50% similarity to SEQ ID NO.1)	
	L I G Y N N Q F S G * A	SEQ ID NO:13
	:         :	
30	F I Q G Y S D L F G N - A	SEQ ID NO:1
	197 kDa (42% identity & 58% similarity SEQ ID NO.2)	
	M Q N S Q T F S V G E L	SEQ ID NO.14
	:     : :	
35	M Q D S P E V S I T T L	SEQ ID NO.2

#### Example 8

Construction of a cosmid library of *Photorhabdus luminescens* W-14 genomic DNA and its screening to isolate genes encoding peptides comprising the toxic protein preparation

As a prerequisite for the production of *Photorhabdus* insect toxic proteins in heterologous hosts, and for other uses, it is necessary to isolate and characterize the genes that encode those

peptides. This objective was pursued in parallel. One approach, described later, was based on the use of monoclonal and polyclonal antibodies raised against the purified toxin which were then used to isolate clones from an expression library. The other approach, described in this example, is based on the use of the N-terminal and internal amino acid sequence data to design degenerate oligonucleotides for use in PCR amplification. Either method can be used to identify DNA clones that contain the peptide-encoding genes so as to permit the isolation of the respective genes, and the determination of their DNA base sequence.

GENOMIC DNA ISOLATION: *Photorhabdus luminescens* strain W-14 (ATCC accession number 55397) was grown on 2% proteose peptone #3 agar (Difco Laboratories, Detroit, MI) and insecticidal toxin competence was maintained by repeated bioassay after passage, using the method described in Example 1 above. A 50 ml shake culture was produced in a 175 ml baffled flask in 2% proteose peptone #3 medium, grown at 28°C and 150 rpm for approximately 24 hours. 15 ml of this culture was pelleted and frozen in its medium at -20°C until it was thawed for DNA isolation. The thawed culture was centrifuged, (700 x g, 30 min) and the floating orange mucopolysaccharide material was removed. The remaining cell material was centrifuged (25,000 x g, 15 min) to pellet the bacterial cells, and the medium was removed and discarded.

Genomic DNA was isolated by an adaptation of the CTAB method described in section 2.4.1 of Current Protocols in Molecular Biology (Ausubel et al. eds, John Wiley & Sons, 1994) [modified to include a salt shock and with all volumes increased 10-fold]. The pelleted bacterial cells were resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) to a final volume of 10 ml, then 12 ml of 5 M NaCl was added; this mixture was centrifuged 20 min at 15,000 x g. The pellet was resuspended in 5.7 ml TE and 300 ml of 10% SDS and 60 ml of 20 mg/ml proteinase K (Gibco BRL Products, Grand Island, NY; in sterile distilled water) were added to the suspension. This mixture was incubated at 37°C for 1 hr; then approximately 10 mg lysozyme (Worthington Biochemical Corp., Freehold, NJ) was added. After an additional 45 min, 1 ml of 5 M NaCl and 800 ml of CTAB/NaCl solution (10% w/v CTAB, 0.7 M

- NaCl) were added. This preparation was incubated 10 min at 65°C, then gently agitated and further incubated and agitated for approximately 20 min to assist clearing of the cellular material. An equal volume of chloroform/isoamyl alcohol solution (24:1, v/v) was added, mixed gently and centrifuged. After two extractions with an equal volume of PCI (phenol/chloroform/isoamyl alcohol; 50:49:1, v/v/v; equilibrated with 1 M Tris-HCl, pH 8.0; Intermountain Scientific Corporation, Kaysville, UT), the DNA was precipitated with 0.6 volume of isopropanol. The DNA precipitate was gently removed with a glass rod, washed twice with 70% ethanol, dried, and dissolved in 2 ml STE (10 mM Tris-HCl pH 8.0, 10 mM NaCl, 1 mM EDTA). This preparation contained 2.5 mg/ml DNA, as determined by optical density at 260 nm (i.e., OD<sub>260</sub>).
- The molecular size range of the isolated genomic DNA was evaluated for suitability for library construction. CHEF gel analysis was performed in 1.5% agarose (Seakem® LE, FMC BioProducts, Rockland, ME) gels with 0.5 X TBE buffer (44.5 mM Tris-HCl pH 8.0, 44.5 mM H<sub>3</sub>BO<sub>3</sub>, 1 mM EDTA) on a BioRad CHEF-DR II apparatus with a Pulsewave 760 Switcher (Bio-Rad Laboratories, Inc., Richmond, CA). The running parameters were: initial A time, 3 sec; final A time, 12 sec; 200 volts; running temperature, 4-18°C; run time, 16.5 hr. Ethidium bromide staining and examination of the gel under ultraviolet light indicated the DNA ranged from 30-250 kbp in size.

- CONSTRUCTION OF LIBRARY: A partial Sau3A 1 digest was made of this *Photorhabdus* genomic DNA preparation. The method was based on section 3.1.3 of Ausubel (*supra.*). Adaptions included running smaller scale reactions under various conditions until nearly optimal results were achieved. Several scaled-up large reactions with varied conditions were run, the results analyzed on CHEF gels, and only the best large scale preparation was carried forward. In the optimal case, 200 µg of *Photorhabdus* genomic DNA was incubated with 1.5 units of Sau3A 1 (New England Biolabs, "NEB", Beverly, MA) for 15 min at 37°C in 2 ml total volume of 1X NEB 4 buffer (supplied as 10X by the manufacturer). The reaction was stopped by adding 2 ml of PCI and centrifuging at 8000 x g for 10 min. To the supernatant were added 200 µl of 5 M NaCl plus 6 ml of ice-cold ethanol. This preparation was

chilled for 30 min at  $-20^{\circ}\text{C}$ , then centrifuged at  $12,000 \times g$  for 15 min. The supernatant was removed and the precipitate was dried in a vacuum oven at  $40^{\circ}\text{C}$ , then resuspended in 400  $\mu\text{l}$  STE. Spectrophotometric assay indicated about 40% recovery of the input DNA. The digested DNA was size fractionated on a sucrose gradient according to section 5.3.2 of CPMB (op. cit.). A 10% to 40% (w/v) linear sucrose gradient was prepared with a gradient maker in Ultra-Clear™ tubes (Beckman Instruments, Inc., Palo Alto, CA) and the DNA sample was layered on top. After centrifugation, (26,000 rpm, 17 hr, Beckman SW41 rotor,  $20^{\circ}\text{C}$ ), fractions (about 750  $\mu\text{l}$ ) were drawn from the top of the gradient and analyzed by CHEF gel electrophoresis (as described earlier). Fractions containing Sau3A I fragments in the size range 20-40 kbp were selected and DNA was precipitated by a modification (amounts of all solutions increased approximately 6.3-fold) of the method in section 5.3.3 of Ausubel (supra.). After overnight precipitation, the DNA was collected by centrifugation ( $17,000 \times g$ , 15 min), dried, redissolved in TE, pooled into a final volume of 80  $\mu\text{l}$ , and reprecipitated with the addition of 8  $\mu\text{l}$  3 M sodium acetate and 220  $\mu\text{l}$  ethanol. The pellet collected by centrifugation as above was resuspended in 12  $\mu\text{l}$  TE. Concentration of the DNA was determined by Hoechst 33258 dye (Polysciences, Inc., Warrington, PA) fluorometry in a Hoefer TKO100 fluorimeter (Hoefer Scientific Instruments, San Francisco, CA). Approximately 2.5  $\mu\text{g}$  of the size-fractionated DNA was recovered.

Thirty  $\mu\text{g}$  of cosmid pWE15 DNA (Stratagene, La Jolla, CA) was digested to completion with 100 units of restriction enzyme BamH I (NEB) in the manufacturer's buffer (final volume of 200  $\mu\text{l}$ ,  $37^{\circ}\text{C}$ , 1 hr). The reaction was extracted with 100  $\mu\text{l}$  of PCI and DNA was precipitated from the aqueous phase by addition of 20  $\mu\text{l}$  3M sodium acetate and 550  $\mu\text{l}$   $-20^{\circ}\text{C}$  absolute ethanol. After 20 min at  $-70^{\circ}\text{C}$ , the DNA was collected by centrifugation ( $17,000 \times g$ , 15 min), dried under vacuum, and dissolved in 180  $\mu\text{l}$  of 10 mM Tris-HCl, pH 8.0. To this were added 20  $\mu\text{l}$  of 10X CIP buffer (100 mM Tris-HCl, pH 8.3; 10 mM  $\text{ZnCl}_2$ ; 10 mM  $\text{MgCl}_2$ ), and 1  $\mu\text{l}$  (0.25 units) of 1:4 diluted calf intestinal alkaline phosphatase

(Boehringer Mannheim Corporation, Indianapolis, IN). After 30 min at 37°C, the following additions were made: 2 µl 0.5 M EDTA, pH 8.0; 10 µl 10% SDS; 0.5 µl of 20 mg/ml proteinase K (as above), followed by incubation at 55°C for 30 min. Following sequential extractions with 100 µl of PCI and 100 µl phenol (Intermountain Scientific Corporation, equilibrated with 1 M Tris-HCl, pH 8.0), the dephosphorylated DNA was precipitated by addition of 72 µl of 7.5 M ammonium acetate and 550 µl -20°C ethanol, incubation on ice for 30 min, and centrifugation as above. The pelleted DNA was washed once with 500 µl -20°C 70% ethanol, dried under vacuum, and dissolved in 20 µl of TE buffer.

Ligation of the size-fractionated Sau3A 1 fragments to the BamH 1-digested and phosphatased pWE15 vector was accomplished using T4 ligase (NEB) by a modification (i.e., use of premixed 10X ligation buffer supplied by the manufacturer) of the protocol in section 3.33 of Ausubel. Ligation was carried out overnight in a total volume of 20 µl at 15°C, followed by storage at -20°C.

Four µl of the cosmid DNA ligation reaction, containing about 1 µg of DNA, was packaged into bacteriophage lambda using a commercial packaging extract (Gigapack<sup>®</sup> III Gold Packaging Extract, Stratagene), following the manufacturer's directions. The packaged preparation was stored at 4°C until use. The packaged cosmid preparation was used to infect *Escherichia coli* XL1 Blue MR cells (Stratagene) according to the Gigapack<sup>®</sup> III Gold protocols ("Titering the Cosmid Library"), as follows. XL1 Blue MR cells were grown in LB medium (g/L: Bacto-tryptone, 10; Bacto-yeast extract, 5; Bacto-agar, 15; NaCl, 5; [Difco Laboratories, Detroit, MI]) containing 0.2% (w/v) maltose plus 10 mM MgSO<sub>4</sub>, at 37°C. After 5 hr growth, cells were pelleted at 700 x g (15 min) and resuspended in 6 ml of 10 mM MgSO<sub>4</sub>. The culture density was adjusted with 10 mM MgSO<sub>4</sub> to OD<sub>600</sub> = 0.5. The packaged cosmid library was diluted 1:10 or 1:20 with sterile SM medium (0.1 M NaCl, 10 mM MgSO<sub>4</sub>, 50 mM Tris-HCl pH 7.5, 0.01% w/v gelatin), and 25 µl of the diluted preparation was mixed with 25 µl of the diluted XL1 Blue MR cells. The mixture was incubated at 25°C for 30 min (without shaking), then 200 µl of LB broth was added, and incubation was continued for approximately 1 hr with occasional

gentle shaking. Aliquots (20-40  $\mu$ l) of this culture were spread on LB agar plates containing 100 mg/l ampicillin (i.e., LB-Amp<sub>100</sub>) and incubated overnight at 37°C. To store the library without amplification, single colonies were picked and inoculated into individual wells of sterile 96-well microwell plates; each well containing 75  $\mu$ l of Terrific Broth (TB media: 12 g/l Bacto-tryptone, 24 g/l Bacto-yeast extract, 0.4% v/v glycerol, 17 mM KH<sub>2</sub>PO<sub>4</sub>, 72 mM K<sub>2</sub>HPO<sub>4</sub>) plus 100 mg/l ampicillin (i.e., TB-Amp<sub>100</sub>) and incubated (without shaking) overnight at 37°C. After replicating the 96-well plate into a copy plate, 75  $\mu$ l/well of filter-sterilized TB:glycerol (1:1, v/v; with, or without, 100 mg/l ampicillin) was added to the plate, it was shaken briefly at 100 rpm, 37°C, and then closed with Parafilm<sup>®</sup> (American National Can, Greenwich, CT) and placed in a -70°C freezer for storage. Copy plates were grown and processed identically to the master plates. A total of 40 such master plates (and their copies) were prepared.

SCREENING OF THE LIBRARY WITH RADIOLABELED DNA PROBES: To prepare colony filters for probing with radioactively labeled probes, ten 96-well plates of the library were thawed at 25°C (bench top at room temperature). A replica plating tool with 96 prongs was used to inoculate a fresh 96-well copy plate containing 75  $\mu$ l/well of TB-Amp<sub>100</sub>. The copy plate was grown overnight (stationary) at 37°C, then shaken about 30 min at 100 rpm at 37°C. A total of 800 colonies was represented in these copy plates, due to nongrowth of some isolates. The replica tool was used to inoculate duplicate impressions of the 96-well arrays onto Magna NT (MSI, Westboro, MA) nylon membranes (0.45 micron, 220 x 250 mm) which had been placed on solid LB-Amp<sub>100</sub> (100 ml/dish) in Bio-assay plastic dishes (Nunc, 243 x 243 x 18 mm; Curtin Mathison Scientific, Inc., Wood Dale, IL). The colonies were grown on the membranes at 37°C for about 3 hr.

A positive control colony (a bacterial clone containing a GZ4 sequence insert, see below) was grown on a separate Magna NT membrane (Nunc, 0.45 micron, 82 mm circle) on LB medium supplemented with 35 mg/l chloramphenicol (i.e., LB-Cam<sub>35</sub>), and processed alongside the library colony membranes. Bacterial colonies on the membranes were lysed, and the DNA was denatured

and neutralized according to a protocol taken from the Genius™ System User's Guide version 2.0 (Boehringer Mannheim, Indianapolis, IN). Membranes were placed colony side up on filter paper soaked with 0.5 N NaOH plus 1.5 M NaCl for 15 min to denature, and neutralized on filter paper soaked with 1 M Tris-HCl pH 8.0, 1.5 M NaCl for 15 min. After UV-crosslinking using a Stratagene UV Stratalinker set on auto crosslink, the membranes were stored dry at 25°C until use. Membranes were trimmed into strips containing the duplicate impressions of a single 96-well plate, then washed extensively by the method of section 6.4.1 in CPMB (op. cit.): 3 hr at 25°C in 3X SSC, 0.1% (w/v) SDS, followed by 1 hr at 65°C in the same solution, then rinsed in 2X SSC in preparation for the hybridization step (20X SSC = 3 M NaCl, 0.3 M sodium citrate, pH 7.0).

#### Amplification of a specific genomic fragment of a tcaC gene.

Based on the N-terminal amino acid sequence determined for the purified TcaC peptide fraction [disclosed herein as SEQ ID NO:2], a pool of degenerate oligonucleotides (pool S4Psh) was synthesized by standard  $\beta$ -cyanoethyl chemistry on an Applied BioSystem ABI394 DNA/RNA Synthesizer (Perkin Elmer, Foster City, CA). The oligonucleotides were deprotected 8 hours at 55°C, dissolved in water, quantitated by spectrophotometric measurement, and diluted for use. This pool corresponds to the determined N-terminal amino acid sequence of the TcaC peptide. The determined amino acid sequence and the corresponding degenerate DNA sequence are given below, where A, C, G, and T are the standard DNA bases, and I represents inosine:

Amino Acid	Met	Gln	Asp	Ser	Pro	Glu	Val
S4Psh	5' ATG	CA(A/G)	GA(T/C)	(T/A)(C/G)(T/A)	CCI	GA(A/G)	GT 3'

Another set of degenerate oligonucleotides was synthesized (pool P2.3.5R), representing the complement of the coding strand for the determined amino acid sequence of the SEQ ID NO:17:

Amino Acid	Ala	Phe	Asn	Ile	Asp	Asp	Val
Codons	5' GCN	TT(T/C)	AA(T/C)	AT(A/T/C)	GA(T/C)	GA(T/C)	GT 3'
P2.3.5R	3'CG(A/C/G/T)	AA(A/G)	TT(A/G)	TA(T/A/G)	CT(A/G)	CT(A/G)	CA 5'

These oligonucleotides were used as primers in Polymerase Chain Reactions (PCR®, Roche Molecular Systems, Branchburg, NJ) to

amplify a specific DNA fragment from genomic DNA prepared from *Photorhabdus* strain W-14 (see above). A typical reaction (50  $\mu$ l) contained 125 pmol of each primer pool P2Psh and P2.3.5R, 253 ng of genomic template DNA, 10 nmol each of dATP, dCTP, dGTP, and dTTP, 1X GeneAmp<sup>®</sup> PCR buffer, and 2.5 units of AmpliTaq<sup>®</sup> DNA polymerase (both from Roche Molecular Systems; 10X GeneAmp<sup>®</sup> buffer is 100 mM Tris-HCl pH 8.3, 500 mM KCl, 0.01% w/v gelatin). Amplifications were performed in a Perkin Elmer Cetus DNA Thermal Cycler (Perkin Elmer, Foster City, CA) using 35 cycles of 94°C (1.0 min), 55°C (2.0 min), 72°C (3.0 min), followed by an extension period of 7.0 min at 72°C. Amplification products were analyzed by electrophoresis through 2% w/v NuSieve<sup>®</sup> 3:1 agarose (FMC BioProducts) in TEA buffer (40 mM Tris-acetate, 2 mM EDTA, pH 8.0). A specific product of estimated size 250 bp was observed amongst numerous other amplification products by ethidium bromide (0.5  $\mu$ g/ml) staining of the gel and examination under ultraviolet light.

The region of the gel containing an approximately 250 bp product was excised, and a small plug (0.5 mm dia.) was removed and used to supply template for PCR amplification (40 cycles). The reaction (50  $\mu$ l) contained the same components as above, minus genomic template DNA. Following amplification, the ends of the fragments were made blunt and were phosphorylated by incubation at 25°C for 20 min with 1 unit of T4 DNA polymerase (NEB), 1 nmol ATP, and 2.15 units of T4 kinase (Pharmacia Biotech Inc., Piscataway, NJ).

DNA fragments were separated from residual primers by electrophoresis through 1% w/v GTG<sup>®</sup> agarose (FMC) in TEA. A gel slice containing fragments of apparent size 250 bp was excised, and the DNA was extracted using a Qiaex kit (Qiagen Inc., Chatsworth, CA).

The extracted DNA fragments were ligated to plasmid vector pBC KS(+) (Stratagene) that had been digested to completion with restriction enzyme Sma I and extracted in a manner similar to that described for pWE15 DNA above. A typical ligation reaction (16.3  $\mu$ l) contained 100 ng of digested pBC KS(+) DNA, 70 ng of 250 bp fragment DNA, 1 nmol [Co(NH<sub>3</sub>)<sub>6</sub>]Cl<sub>2</sub>, and 3.9 Weiss units of T4 DNA ligase (Collaborative Biomedical Products, Bedford, MA), in 1X ligation buffer (50 mM Tris-HCl, pH 7.4; 10 mM MgCl<sub>2</sub>; 10 mM



dithiothreitol; 1 mM spermidine, 1 mM ATP, 100 mg/ml bovine serum albumin). Following overnight incubation at 14°C, the ligated products were transformed into frozen, competent *Escherichia coli* DH5α cells (Gibco BRL) according to the suppliers' recommendations, and plated on LB-Cam<sub>3</sub> plates, containing IPTG (119 µg/ml) and X-gal (50 µg/ml). Independent white colonies were picked, and plasmid DNA was prepared by a modified alkaline-lysis/PEG precipitation method (PRISM™ Ready Reaction DyeDeoxy™ Terminator Cycle Sequencing Kit Protocols; ABI/Perkin Elmer).

10 The nucleotide sequence of both strands of the insert DNA was determined, using T7 primers [pBC KS(+) bases 601-623: TAAAACGACGCCAGTGAGCGCG) and LacZ primers [pBC KS(+) bases 792-816: ATGACCATGATTACGCCAAGCGCGC) and protocols supplied with the PRISM™ sequencing kit (ABI/Perkin Elmer). Nonincorporated dye-

15 terminator dideoxyribonucleotides were removed by passage through Centri-Sep 100 columns (Princeton Separations, Inc., Adelphia, NJ) according to the manufacturer's instructions. The DNA sequence was obtained by analysis of the samples on an ABI Model 373A DNA Sequencer (ABI/Perkin Elmer). The DNA sequences of two

20 isolates, GZ4 and HB14, were found to be as illustrated in Figure 1.

This sequence illustrates the following features: 1) bases 1-20 represent one of the 64 possible sequences of the S4Psh degenerate oligonucleotides, ii) the sequence of amino acids 1-3 and 6-12 correspond exactly to that determined for the N-terminus of TcaC (disclosed as SEQ ID NO:2), iii) the fourth amino acid encoded is a cysteine residue rather than serine. This difference is encoded within the degeneracy for the serine codons (see above), iv) the fifth amino acid encoded is proline,

25 corresponding to the TcaC N-terminal sequence given as SEQ ID NO:2, v) bases 257-276 encode one of the 192 possible sequences designed into the degenerate pool, vi) the TGA termination codon introduced at bases 268-270 is the result of complementarity to the degeneracy built into the oligonucleotide pool at the

30 corresponding position, and does not indicate a shortened reading frame for the corresponding gene.

Labeling of a TcaC peptide gene-specific probe. DNA fragments corresponding to the above 276 bases were amplified (35

cycles) by PCR<sup>®</sup> in a 100 µl reaction volume, using 100 pmol each of P2Psh and P2.3.5R primers, 10 ng of plasmids GZ4 or HB14 as templates, 20 nmol each of dATP, dCTP, dGTP, and dTTP, 5 units of AmpliTaq<sup>®</sup> DNA polymerase, and 1X concentration of GeneAmp<sup>®</sup> buffer, under the same temperature regimes as described above. The amplification products were extracted from a 1% GTG<sup>®</sup> agarose gel by Qiaex kit and quantitated by fluorometry.

The extracted amplification products from plasmid HB14 template (approximately 400 ng) were split into five aliquots and labeled with <sup>32</sup>P-dCTP using the High Prime Labeling Mix (Boehringer Mannheim) according to the manufacturer's instructions. Nonincorporated radioisotope was removed by passage through NucTrap<sup>®</sup> Probe Purification Columns (Stratagene), according to the supplier's instructions. The specific activity of the labeled DNA product was determined by scintillation counting to be  $3.11 \times 10^8$  dpm/µg. This labeled DNA was used to probe membranes prepared from 800 members of the genomic library.

Screening with a TcaC-peptide gene specific probe. The radiolabeled HB14 probe was boiled approximately 10 min, then added to "minimal hyb" solution. [Note: The "minimal hyb" method is taken from a CERES protocol; "Restriction Fragment Length Polymorphism Laboratory Manual version 4.0", sections 4-40 and 4-47; CERES/NPI, Salt Lake City, UT. NPI is now defunct, with its successors operating as Linkage Genetics]. "Minimal hyb" solution contains 10% w/v PEG (polyethylene glycol, M.W. approx. 8000), 7% w/v SDS, 0.6X SSC, 10 mM sodium phosphate buffer (from a 1M stock containing 95 g/l NaH<sub>2</sub>PO<sub>4</sub>·1H<sub>2</sub>O and 84.5 g/l Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O), 5 mM EDTA, and 100 mg/ml denatured salmon sperm DNA. Membranes were blotted dry briefly then, without prehybridization, 5 strips of membrane were placed in each of 2 plastic boxes containing 75 ml of "minimal hyb" and 2.6 ng/ml of radiolabeled HB14 probe. These were incubated overnight with slow shaking (50 rpm) at 60°C. The filters were washed three times for approximately 10 min each at 25°C in "minimal hyb wash solution" (0.25X SSC, 0.2% SDS), followed by two 30-min washes with slow shaking at 60°C in the same solution. The filters were placed on paper covered with Saran Wrap<sup>®</sup> (Dow Brands, Indianapolis, IN) in a light-tight autoradiographic cassette and exposed to X-Omat X-ray film (Kodak, Rochester, NY) with two

DuPont Cronex Lightning-Plus C1 enhancers (Sigma Chemical Co., St. Louis, MO), for 4 hr at -70°C. Upon development (standard photographic procedures), significant signals were evident in both replicates amongst a high background of weaker, more irregular signals. The filters were again washed for about 4 hr at 68°C in "minimal hyb wash solution" and then placed again in the cassettes and film was exposed overnight at -70°C. Twelve possible positives were identified due to strong signals on both of the duplicate 96-well colony impressions. No signal was seen with negative control membranes (colonies of XL1 Blue MR cells containing pWE15), and a very strong signal was seen with positive control membranes (DH5α cells containing the GZ4 isolate of the PCR product) that had been processed concurrently with the experimental samples.

The twelve putative hybridization-positive colonies were retrieved from the frozen 96-well library plates and grown overnight at 37°C on solid LB-Amp<sub>100</sub> medium. They were then patched (3/plate, plus three negative controls: XL1 Blue MR cells containing the pWE15 vector) onto solid LB-Amp<sub>100</sub>. Two sets of membranes (Magna NT nylon, 0.45 micron) were prepared for hybridization. The first set was prepared by placing a filter directly onto the colonies on a patch plate, then removing it with adherent bacterial cells, and processing as below. Filters of the second set were placed on plates containing LB-Amp<sub>100</sub> medium, then inoculated by transferring cells from the patch plates onto the filters. After overnight growth at 37°C, the filters were removed from the plates and processed.

Bacterial cells on the filters were lysed and DNA denatured by placing each filter colony-side-up on a pool (1.0 ml) of 0.5 N NaOH in a plastic plate for 3 min. The filters were blotted dry on a paper towel, then the process was repeated with fresh 0.5 N NaOH. After blotting dry, the filters were neutralized by placing each on a 1.0 ml pool of 1 M Tris-HCl, pH 7.5 for 3 min, blotted dry, and reneutralised with fresh buffer. This was followed by two similar soakings (5 min each) on pools of 0.5 M Tris-HCl pH 7.5 plus 1.5 M NaCl. After blotting dry, the DNA was UV crosslinked to the filter (as above), and the filters were washed (25°C, 100 rpm) in about 100 ml of 3X SSC plus 0.1% (w/v) SDS (4 times, 30 min each with fresh solution for each wash). They were then placed in a minimal volume of prehybridization

solution [6X SSC plus 1% w/v each of Ficoll 400 (Pharmacia), polyvinylpyrrolidone (av. M.W. 360,000; Sigma) and bovine serum albumin Fraction V; (Sigma)] for 2 hr at 65°C, 50 rpm. The prehybridization solution was removed, and replaced with the HB14 <sup>32</sup>P-labeled probe that had been saved from the previous hybridization of the library membranes and which had been denatured at 95°C for 5 min. Hybridization was performed at 60°C for 16 hr with shaking at 50 rpm.

Following removal of the labeled probe solution, the membranes were washed 3 times at 25°C (50 rpm, 15 min) in 3X SSC (about 150 ml each wash). They were then washed for 3 hr at 68°C (50 rpm) in 0.25X SSC plus 0.2% SDS (minimal hyb wash solution), and exposed to X-ray film as described above for 1.5 hr at 25°C (no enhancer screens). This exposure revealed very strong hybridization signals to cosmid isolates 22G12, 25A10, 26A5, and 26B10, and a very weak signal with cosmid isolate 8B10. No signal was seen with the negative control (pWE15) colonies, and a very strong signal was seen with positive control membranes (DH5α cells containing the GZ4 isolate of the PCR product) that had been processed concurrently with the experimental samples.

#### Amplification of a specific genomic fragment of a *tcaB* gene.

Based on the N-terminal amino acid sequence determined for the purified TcaB<sub>i</sub> peptide fraction (disclosed here as SEQ ID NO:3) a pool of degenerate oligonucleotides (pool P8F) was synthesized as described for peptide TcaC. The determined amino acid sequence and the corresponding degenerate DNA sequence are given below, where A, C, G, and T are the standard DNA bases, and I represents inosine:

30	Amino										
	Acid	Leu	Phe	Thr	Gln	Thr	Leu	Lys	Glu	Ala	Arg
35	P8F	5'	TTT	ACI	CA(A/G)	ACI	(C/T)TI	AAA	GAA	GCI	(A/C)G 3'
			(C/T)TI								

Another set of degenerate oligonucleotides was synthesized (pool P8.108.3R), representing the complement of the coding strand for the determined amino acid sequence of the TcaB<sub>i</sub>-PT108 internal peptide (disclosed herein as SEQ ID NO:20):

Amino								
Acid	Met	Tyr	Tyr	Ile	Gln	Ala	Gln	Gln

Codons ATG TA(T/C) TA(T/C) AT(T/C/A) CA(A/G) GC(A/C/G/T) CA(A/G) CA(A/G)  
 P8.108.3R 3' AT(A/G) AT(A/G) TA(A/G/T) GT(T/C) CGI GT(T/C) GT 5'  
 TAC

5

These oligonucleotides were used as primers for PCR<sup>®</sup> using HotStart 50 Tubes<sup>™</sup> (Molecular Bio-Products, Inc., San Diego, CA) to amplify a specific DNA fragment from genomic DNA prepared from *Photorhabdus* strain W-14 (see above). A typical reaction (50  $\mu$ l) contained (bottom layer) 25 pmol of each primer pool P8F and P8.108.3R, with 2 nmol each of dATP, dCTP, dGTP, and dTTP, in 1X GeneAmp<sup>®</sup> PCR buffer, and (top layer) 230 ng of genomic template DNA, 8 nmol each of dATP, dCTP, dGTP, and dTTP, and 2.5 units of AmpliTaq<sup>®</sup> DNA polymerase, in 1X GeneAmp<sup>®</sup> PCR buffer.

Amplifications were performed by 35 cycles as described for the TcaC peptide. Amplification products were analyzed by electrophoresis through 0.7% w/v SeaKem<sup>®</sup> LE agarose (FMC) in TEA buffer. A specific product of estimated size 1600 bp was observed.

Four such reactions were pooled, and the amplified DNA was extracted from a 1.0% SeaKem<sup>®</sup> LE gel by Qiaex kit as described for the TcaC peptide. The extracted DNA was used directly as the template for sequence determination (PRISM<sup>™</sup> Sequencing Kit) using the P8F and P8.108.3R primer pools. Each reaction contained about 100 ng template DNA and 25 pmol of one primer pool, and was processed according to standard protocols as described for the TcaC peptide. An analysis of the sequence derived from extension of the P8F primers revealed the short DNA sequence (and encoded amino acid sequence):

GAT GCA TTG NTT GCT  
 Asp Ala Leu (Val) Ala

which corresponds to a portion of the N-terminal peptide sequence disclosed as SEQ ID NO:3 (TcaB<sub>i</sub>).

### 35 Labeling of a TcaB<sub>i</sub>-peptide gene-specific probe.

Approximately 50 ng of gel-purified TcaB<sub>i</sub> DNA fragment was labeled with <sup>32</sup>P-dCTP as described above, and nonincorporated radioisotopes were removed by passage through a NICK Column<sup>®</sup> (Pharmacia). The specific activity of the labelled DNA was determined to be 6 x 10<sup>9</sup> dpm/ $\mu$ g. This labeled DNA was used to

probe colony membranes prepared from members of the genomic library that had hybridized to the TcaC-peptide specific probe.

The membranes containing the 12 colonies identified in the TcaC-probe library screen (see above) were stripped of  
5 radioactive TcaC-specific label by boiling twice for approximately 30 min each time in 1 liter of 0.1X SSC plus 0.1 % SDS. Removal of radiolabel was checked with a 6 hr film exposure. The stripped membranes were then incubated with the TcaB<sub>i</sub> peptide-specific probe prepared above. The labeled DNA was  
10 denatured by boiling for 10 min, and then added to the filters that had been incubated for 1 hr in 100 ml of "minimal hyb" solution at 60°C. After overnight hybridization at this temperature, the probe solution was removed, and the filters were washed as follows (all in 0.3X SSC plus 0.1% SDS): once for 5 min  
15 at 25°C, once for 1 hr at 60°C in fresh solution, and once for 1 hr at 63°C in fresh solution. After 1.5 hr exposure to X-ray film by standard procedures, 4 strongly-hybridizing colonies were observed. These were, as with the TcaC-specific probe, isolates 22G12, 25A10, 26A5, and 26B10.

20 The same TcaB<sub>i</sub> probe solution was diluted with an equal volume (about 100 ml) of "minimal hyb" solution, and then used to screen the membranes containing the 800 members of the genomic library. After hybridization, washing, and exposure to X-ray film as described above, only the four cosmid clones 22G12,  
25 25A10, 26A5, and 26B10, were found to hybridize strongly to this probe.

ISOLATION OF SUBCLONES CONTAINING GENES ENCODING TcaC AND TcaB<sub>i</sub> PEPTIDES, AND DETERMINATION OF DNA BASE SEQUENCE THEREOF:

30 Three hybridization-positive cosmids in strain XL1 Blue MR were grown with shaking overnight (200 rpm) at 30°C in 100 ml TB-Amp<sub>100</sub>. After harvesting the cells by centrifugation, cosmid DNA was prepared using a commercially available kit (BIGprep™, 5 Prime 3 Prime, Inc., Boulder, CO), following the manufacturer's  
35 protocols. Only one cosmid, 26A5, was successfully isolated by this procedure. When digested with restriction enzyme EcoR 1 (NEB) and analyzed by gel electrophoresis, fragments of approximate sizes 14, 10, 8 (vector), 5, 3.3, 2.9, and 1.5 kbp were detected. A second attempt to isolate cosmid DNA from the  
40 same three strains (8 ml cultures; TB-Amp<sub>100</sub>, 30°C) utilized a

boiling miniprep method (Evans G. and G. Wahl., 1987, "Cosmid vectors for genomic walking and rapid restriction mapping." in Guide to Molecular Cloning Techniques, Meth. Enzymology, vol. 152, S. Berger and A. Kimmel, eds., pgs. 604-610). Only one  
5 cosmid, 25A10, was successfully isolated by this method. When digested with restriction enzyme EcoR 1 (NEB) and analyzed by gel electrophoresis, this cosmid showed a fragmentation pattern identical to that previously seen with cosmid 26A5.

A 0.15 µg sample of 26A5 cosmid DNA was used to transform 50  
10 ml of *E. coli* DH5α cells (Gibco BRL), by the supplier's protocols. A single colony isolate of that strain was inoculated into 4 ml of TB-Amp<sub>100</sub>, and grown for 8 hr at 37°C. Chloramphenicol was added to a final concentration of 225 µg/ml, incubation was continued for another 24 hr, then cells were  
15 harvested by centrifugation and frozen at -20°C. Isolation of the 26A5 cosmid DNA was by a standard alkaline lysis miniprep (Maniatis et al., op. cit., p. 382), modified by increasing all volumes by 50% and with stirring or gentle mixing, rather than vortexing, at every step. After washing the DNA pellet in 70%  
20 ethanol, it was dissolved in TE containing 25 µg/ml ribonuclease A (Boehringer Mannheim).

Identification of EcoR 1 fragments hybridizing to GZ4-  
derived and TcaB<sub>1</sub>- probes. Approximately 0.4 µg of cosmid 25A10  
25 (from XL1 Blue MR cells) and about 0.5 µg of cosmid 26A5 (from chloramphenicol-amplified DH5α cells) were each digested with about 15 units of EcoR 1 (NEB) for 85 min, frozen overnight, then heated at 65°C for five min, and electrophoresed in a 0.7% agarose gel (Seakem® LE, 1X TEA, 80 volts, 90 min). The DNA was  
30 stained with ethidium bromide as described above, and photographed under ultraviolet light. The EcoR 1 digest of cosmid 25A10 was a complete digestion, but the sample of cosmid 26A5 was only partially digested under these conditions. The agarose gel containing the DNA fragments was subjected to  
35 depurination, denaturation and neutralization, followed by Southern blotting onto a Magna NT nylon membrane, using a high salt (20X SSC) protocol, all as described in section 2.9 of Ausubel et al. (CPMB, op. cit.). The transferred DNA was then UV-crosslinked to the nylon membrane as before.

An TcaC-peptide specific DNA fragment corresponding to the insert of plasmid isolate GZ4 was amplified by PCR<sup>®</sup> in a 100 ml reaction volume as described previously above. The amplification products from three such reactions were pooled and were extracted from a 1% GTG<sup>®</sup> agarose gel by Qiaex kit, as described above, and quantitated by fluorometry. The gel-purified DNA (100 ng) was labeled with <sup>32</sup>P-dCTP using the High Prime Labeling Mix (Boehringer Mannheim) as described above, to a specific activity of  $6.34 \times 10^8$  dpm/ $\mu$ g.

10        The <sup>32</sup>P-labeled GZ4 probe was boiled 10 min, then added to "minimal hyb" buffer (at 1 ng/ml), and the Southern blot membrane containing the digested cosmid DNA fragments was added, and incubated for 4 hr at 60°C with gentle shaking at 50 rpm. The membrane was then washed 3 times at 25°C for about 5 min each (minimal hyb wash solution), followed by two washes for 10 min each at 60°C. The blot was exposed to film (with enhancer screens) for about 30 min at -70°C. The GZ4 probe hybridized strongly to the 5.0 kbp (apparent size) EcoR 1 fragment of both these two cosmids, 26A5 and 25A10.

20        The membrane was stripped of radioactivity by boiling for about 30 min in 0.1X SSC plus 0.1 % SDS, and absence of radiolabel was checked by exposure to film. It was then hybridized at 60°C for 3.5 hours with the (denatured) TcaB<sub>i</sub> probe in "minimal hyb" buffer previously used for screening the colony membranes (above), washed as described previously, and exposed to film for 40 min at -70°C with two enhancer screens. With both cosmids, the TcaB<sub>i</sub> probe hybridized lightly with the about 5.0 kbp EcoR 1 fragment, and strongly with a fragment of approximately 2.9 kbp.

30        The sample of cosmid 26A5 DNA previously described, (from DH5 $\alpha$  cells) was used as the source of DNA from which to subclone the bands of interest. This DNA (2.5  $\mu$ g) was digested with about 3 units of EcoR 1 (NEB) in a total volume of 30  $\mu$ l for 1.5 hr, to give a partial digest, as confirmed by gel electrophoresis. Ten  $\mu$ g of pBC KS (+) DNA (Stratagene) were digested for 1.5 hr with 20 units of EcoR 1 in a total volume of 20  $\mu$ l, leading to total digestion as confirmed by electrophoresis. Both EcoR 1-cut DNA preparations were diluted to 50  $\mu$ l with water, to each an equal volume of PCI was added, the suspension was gently mixed, spun in



a microcentrifuge and the aqueous supernatant was collected. DNA was precipitated by 150 µl ethanol, and the mixture was placed at -20°C overnight. Following centrifugation and drying, the EcoR 1-digested pBC KS (+) was dissolved in 100 µl TE; the partially digested 26A5 was dissolved in 20 µl TE. DNA recovery was checked by fluorometry.

In separate reactions, approximately 60 ng of EcoR 1-digested pBC KS(+) DNA was ligated with approximately 180 ng or 270 ng of partially digested cosmid 26A5 DNA. Ligations were carried out in a volume of 20 µl at 15°C for 5 hr, using T4 ligase and buffer from New England BioLabs. The ligation mixture, diluted to 100 µl with sterile TE, was used to transform frozen, competent DH5α cells (Gibco BRL) according to the supplier's instructions. Varying amounts (25-200 µl) of the transformed cells were plated on freshly prepared solid LB-Cam<sub>r</sub>s medium with 1 mM IPTG and 50 mg/l X-gal. Plates were incubated at 37°C about 20 hr, then chilled in the dark for approximately 3 hr to intensify color for insert selection. White colonies were picked onto patch plates of the same composition and incubated overnight at 37°C.

Two colony lifts of each of the selected patch plates were prepared as follows. After picking white colonies to fresh plates, round Magna NT nylon membranes were pressed onto the patch plates, the membrane was lifted off, and subjected to denaturation, neutralization and UV crosslinking as described above for the library colony membranes. The crosslinked colony lifts were vigorously washed, including gently wiping off the excess cell debris with a tissue. One set was hybridized with the GZ4(TcaC) probe solution described earlier, and the other set was hybridized with the TcaB<sub>i</sub> probe solution described earlier, according to the 'minimal hyb' protocol, followed by washing and film exposure as described for the library colony membranes.

Colonies showing hybridization signals either only with the GZ4 probe, with both GZ4 and TcaB<sub>i</sub> probes, or only with the TcaB<sub>i</sub> probe, were selected for further work and cells were streaked for single colony isolation onto LB-Cam<sub>r</sub>s media with IPTG and X-gal as before. Approximately 35 single colonies, from 16 different isolates, were picked into liquid LB-Cam<sub>r</sub>s media and grown

overnight at 37°C; the cells were collected by centrifugation and plasmid DNA was isolated by a standard alkaline lysis miniprep according to Maniatis *et al.* (*op. cit.* p. 368). DNA pellets were dissolved in TE + 25 µg/ml ribonuclease A and DNA concentration was determined by fluorometry. The EcoR 1 digestion pattern was analyzed by gel electrophoresis. The following isolates were picked as useful. Isolate A17.2 contains religated pBC KS(+) only and was used for a (negative) control. Isolates D38.3 and C44.1 each contain only the 2.9 kbp, TcaB<sub>i</sub> -hybridizing EcoR 1 fragment inserted into pBC KS(+). These plasmids, named pDAB2000 and pDAB2001, respectively, are illustrated in Fig. 2.

Isolate A35.3 contains only the approximately 5 kbp, GZ4)-hybridizing EcoR 1 fragment, inserted into pBC KS(+). This plasmid was named pDAB2002 (also Fig. 2). These isolates provided templates for DNA sequencing.

Plasmids pDAB2000 and pDAB2001 were prepared using the BIGprep™ kit as before. Cultures (30 ml) were grown overnight in TB-Cam<sub>35</sub> to an OD<sub>600</sub> of 2, then plasmid was isolated according to the manufacturer's directions. DNA pellets were redissolved in 100 µl TE each, and sample integrity was checked by EcoR 1 digestion and gel electrophoretic analysis.

Sequencing reactions were run in duplicate, with one replicate using as template pDAB2000 DNA, and the other replicate using as template pDAB2001 DNA. The reactions were carried out using the dideoxy dye terminator cycle sequencing method, as described above for the sequencing of the GZ4/HB14 DNAs. Initial sequencing runs utilized as primers the LacZ and T7 primers described above, plus primers based on the determined sequence of the TcaB<sub>i</sub> PCR amplification product (TH1 = ATTGCAGACTGCCAATCGCTTCGG, TH12 = GAGAGTATCCAGACCGCGGATGATCTG).

After alignment and editing of each sequencing output, each was truncated to between 250 to 350 bases, depending on the integrity of the chromatographic data as interpreted by the Perkin Elmer Applied Biosystems Division SeqEd 675 software. Subsequent sequencing "steps" were made by selecting appropriate sequence for new primers. With a few exceptions, primers (synthesized as described above) were 24 bases in length with a 50% G+C composition. Sequencing by this method was carried out on both strands of the approximately 2.9 kbp EcoR 1 fragment.

To further serve as template for DNA sequencing, plasmid DNA from isolate pDAB2002 was prepared by BIGprep™ kit. Sequencing reactions were performed and analyzed as described above.

Initially, a T3 primer (pBS SK (+) bases 774-796:

5 CGCGCAATTAACCCTCACTAAAG) and a T7 primer (pBS KS (+) bases 621-643: GCGCGTAATACGACTCACTATAG) were used to prime the sequencing reactions from the flanking vector sequences, reading into the insert DNA. Another set of primers, (GZ4F:

10 GTATCGATTACAACGCTGTCACTTCCC; TH13: GGAAGTGACAGCGTTGTAATCGATAC; TH14: ATGTTGGGTGCGTCGGCTAATGGACATAAC; and LW1-204:

GGAAGTGACAGCGTTGTAATCGATAC) was made to prime from internal sequences, which were determined previously by degenerate oligonucleotide-mediated sequencing of subcloned TcaC-peptide PCR products. From the data generated during the initial rounds of  
15 sequencing, new sets of primers were designed and used to walk the entire length of the ~5 kbp fragment. A total of 55 oligo primers was used, enabling the identification of 4832 total bp of contiguous sequence.

When the DNA sequence of the EcoR 1 fragment insert of  
20 pDAB2002 is combined with part of the determined sequence of the pDAB2000/pDAB2001 isolates, a total contiguous sequence of 6005 bp was generated (disclosed herein as SEQ ID NO:25). When long open reading frames were translated into the corresponding amino acids, the sequence clearly shows the TcaB<sub>i</sub> N-terminal peptide  
25 (disclosed as SEQ ID NO:3), encoded by bases 19-75, immediately following a methionine residue (start of translation). Upstream lies a potential ribosome binding site (bases 1-9), and downstream, at bases 166-228 is encoded the TcaB<sub>i</sub>-PT158 internal peptide (disclosed herein as SEQ ID NO:19). Further downstream,  
30 in the same reading frame, at bases 1738-1773, exists a sequence encoding the TcaB<sub>i</sub>-PT108 internal peptide (disclosed herein as SEQ ID NO:20). Also in the same reading frame, at bases 1897-1923, is encoded the TcaB<sub>ii</sub> N-terminal peptide (disclosed herein as SEQ ID NO:5), and the reading frame continues uninterrupted to  
35 a translation termination codon at nucleotides 3586-3588.

The lack of an in-frame stop codon between the end of the sequence encoding TcaB<sub>i</sub>-PT108 and the start of the TcaB<sub>ii</sub> encoding region, and the lack of a discernible ribosome binding site immediately upstream of the TcaB<sub>ii</sub> coding region, indicate that

peptides TcaB<sub>ii</sub> and TcaB<sub>i</sub> are encoded by a single open reading frame of 3567 bp beginning at base pair 16 in SEQ ID NO:25), and are most likely derived from a single primary gene product of 1189 amino acids (131,586 Daltons; disclosed herein as SEQ ID NO:26) by post-translational cleavage. If the amino acid immediately preceding the TcaB<sub>ii</sub> N-terminal peptide represents the C-terminal amino acid of peptide TcaB<sub>i</sub>, then the predicted mass of TcaB<sub>ii</sub> (627 amino acids) is 70,814 Daltons (disclosed herein as SEQ ID NO:28), somewhat higher than the size observed by SDS-PAGE (68 kDa). This peptide would be encoded by a contiguous stretch of 1881 base pairs (disclosed herein as SEQ ID NO:27). It is thought that the native C-terminus of TcaB<sub>i</sub> lies somewhat closer to the C-terminus of TcaB<sub>i</sub>-PT108. The molecular mass of PT108 [3.438 kDa; determined during N-terminal amino acid sequence analysis of this peptide] predicts a size of 30 amino acids. Using the size of this peptide to designate the C-terminus of the TcaB<sub>i</sub> coding region [Glu at position 604 of SEQ ID NO:28], the derived size of TcaB<sub>i</sub> is determined to be 604 amino acids or 68,463 Daltons, more in agreement with experimental observations.

Translation of the TcaB<sub>ii</sub> peptide coding region of 1686 base pairs (disclosed herein as SEQ ID NO:29) yields a protein of 562 amino acids (disclosed herein as SEQ ID NO:30) with predicted mass of 60,789 Daltons, which corresponds well with the observed 61 kDa.

A potential ribosome binding site (bases 3633-3638) is found 48 bp downstream of the stop codon for the *tcaB* open reading frame. At bases 3645-3677 is found a sequence encoding the N-terminus of peptide TcaC, (disclosed as SEQ ID NO.2). The open reading frame initiated by this N-terminal peptide continues uninterrupted to base 6005 (2361 base pairs, disclosed herein as the first 2361 base pairs of SEQ ID NO.31). A gene (*tcaC*) encoding the entire TcaC peptide, (apparent size ~165 kDa; ~1500 amino acids), would comprise about 4500 bp.

Another isolate containing cloned EcoR 1 fragments of cosmid 26A5, E20.6, was also identified by its homology to the previously mentioned GZ4 and TcaB<sub>i</sub> probes. Agarose gel analysis of EcoR 1 digests of the DNA of the plasmid harbored by this strain (pDAB2004, Fig. 2), revealed insert fragments of estimated

sizes 2.9, 5, and 3.3 kbp. DNA sequence analysis initiated from primers designed from the sequence of plasmid pDAB2002 revealed that the 3.3 kbp EcoR 1 fragment of pDAB2004 lies adjacent to the 5 kbp EcoR 1 fragment represented in pDAB2002. The 2361 base pair open reading frame discovered in pDAB2002 continues uninterrupted for another 2094 bases in pDAB2004 [disclosed herein as base pairs 2362 to 4458 of SEQ ID NO:31]. DNA sequence analysis using the parent cosmid 26A5 DNA as template confirmed the continuity of the open reading frame. Altogether, the open reading frame (TcaC SEQ ID NO:31) comprises 4455 base pairs, and encodes a protein (TcaC) of 1485 amino acids [disclosed herein as SEQ ID NO:32]. The calculated molecular size of 166,214 Daltons is consistent with the estimated size of the TcaC peptide (165 kDa), and the derived amino acid sequence matches exactly that disclosed for the TcaC N-terminal sequence [SEQ ID NO:2].

The lack of an amino acid sequence corresponding to SEQ ID NO:17; used to design the degenerate oligonucleotide primer pool in the discovered sequence indicates that the generation of the PCR® products found in isolates GZ4 and HB14, which were used as probes in the initial library screen, were fortuitously generated by reverse-strand priming by one of the primers in the degenerate pool. Further, the derived protein sequence does not include the internal fragment disclosed herein as SEQ ID NO:18. These sequences reveal that plasmid pDAB2004 contains the complete coding region for the TcaC peptide.

#### Example 9

##### Screening of the *Photorhabdus* genomic library for genes encoding the TcbA<sub>ii</sub> peptide

30

This example describes a method used to identify DNA clones that contain the TcbA<sub>ii</sub> peptide-encoding genes, the isolation of the gene, and the determination of its partial DNA base sequence.

35

##### Primers and PCR reactions

The TcbA<sub>ii</sub> polypeptide of the insect active preparation is ~206 kDa. The amino acid sequence of the N-terminus of this peptide is disclosed as SEQ ID NO:1. Four pools of degenerate oligonucleotide primers ("Forward primers": TH-4, TH-5, TH-6, and

TH-7) were synthesized to encode a portion of this amino acid sequence, as described in Example 8, and are shown below.

Table 11

5	Amino Acid	Phe	Ile	Gln	Gly	Tyr	Ser	Asp	Leu	Phe
	TH-4	5'-TT(T/C)	ATI	CA(A/G)	GGI	TA(T/C)	TCI	GA(T/C)	CTI	TT-3'
	TH-5	5'-TT(T/C)	ATI	CA(A/G)	GGI	TA(T/C)	AG(T/C)	GA(T/C)	CTI	TT-3'
	TH-6	5'-TT(T/C)	ATI	CA(A/G)	GGI	TA(T/C)	TCI	GA(T/C)	TT(A/G)	TT-3'
10	TH-7	5'-TT(T/C)	ATI	CA(A/G)	GGI	TA(T/C)	AG(T/C)	GA(T/C)	TT(A/G)	TT-3'

In addition, a primary ("a") and a secondary ("b") sequence of an internal peptide preparation (TcbA<sub>ij</sub>-PT81) have been determined and are disclosed herein as SEQ ID No:23 and SEQ ID No:24, respectively. Four pools of degenerate oligonucleotides ("Reverse Primers": TH-8, TH-9, TH-10 and TH-11) were similarly designed and synthesized to encode the reverse complement of sequences that encode a portion of the peptide of SEQ ID NO:23, as shown below.

Table 12

Amino Acid	Thr	Tyr	Leu	Thr	Ser	Phe	Glu	Gln	Val	Ala	Asn
TH-8	3'TGI	AT(A/G)	GAI	TGI	AGI	AA(A/G)	CT(T/C)	GT(T/C)	CAI	CGI	TT(G/A)-5'
TH-9	3'TGI	AT(A/G)	TT(A/G)	TGI	AGI	AA(A/G)	CT(T/C)	GT(T/C)	CAI	CGI	TT(G/A)-5'
TH-10	3'TGI	AT(A/G)	GAI	TGI	TC(G/A)	AA(A/G)	CT(T/C)	GT(T/C)	CAI	CGI	TT(G/A)-5'
TH-11	3'TGI	AT(A/G)	TT(A/G)	TGI	TC(G/A)	AA(A/G)	CT(T/C)	GT(T/C)	CAI	CGI	TT(G/A)-5'

Sets of these primers were used in PCR<sup>®</sup> reactions to amplify TcbA<sub>ii</sub>- encoding gene fragments from the genomic *Photorhabdus luminescens* W-14 DNA prepared in Example 6. All PCR<sup>®</sup> reactions were run with the "Hot Start" technique using AmpliWax<sup>™</sup> gems and other Perkin Elmer reagents and protocols. Typically, a mixture (total volume 11  $\mu$ l) of MgCl<sub>2</sub>, dNTP's, 10X GeneAmp<sup>®</sup> PCR Buffer II, and the primers were added to tubes containing a single wax bead. [10X GeneAmp<sup>®</sup> PCR Buffer II is composed of 100 mM Tris-HCl, pH 8.3; and 500 mM KCl.] The tubes were heated to 80°C for 2 minutes and allowed to cool. To the top of the wax seals, a solution containing 10X GeneAmp<sup>®</sup> PCR Buffer II, DNA template, and AmpliTaq<sup>®</sup> DNA polymerase were added. Following melting of the wax seal and mixing of components by thermal cycling, final reaction conditions (volume of 50  $\mu$ l) were: 10 mM Tris-HCl, pH 8.3; 50 mM KCl; 2.5 mM MgCl<sub>2</sub>; 200  $\mu$ M each in dATP, dCTP, dGTP, dTTP; 1.25 mM in a single Forward primer pool; 1.25  $\mu$ M in a single Reverse primer pool, 1.25 units of AmpliTaq<sup>®</sup> DNA polymerase, and 170 ng of template DNA.

The reactions were placed in a thermocycler (as in Example 8) and run with the following program:

Table 13

Temperature	Time	Cycle Repetition
94°C	2 minutes	1X
94°C	15 seconds	30X
55-65°C	30 seconds	
72°C	1 minute	
72°C	7 minutes	1X
15°C	Constant	



A series of amplifications was run at three different annealing temperatures (55°, 60°, 65° C) using the degenerate primer pools. Reactions with annealing at 65°C had no amplification products visible following agarose gel electrophoresis. Reactions having a 60°C annealing regime and containing primers TH-5+TH-10 produced an amplification product that had a mobility corresponding to 2.9 kbp. A lesser amount of the 2.9 kbp product was produced under these conditions with primers TH-7+TH-10. When reactions were annealed at 55°C, these primer pairs produced more of the 2.9 kbp product, and this product was also produced by primer pairs TH-5+TH-8 and TH-5+TH-11. Additional very faint 2.9 kbp bands were seen in lanes containing amplification products from primer pairs TH-7 plus TH-8, TH-9, TH-10, or TH-11.

To obtain sufficient PCR amplification product for cloning and DNA sequence determination, 10 separate PCR reactions were set up using the primers TH-5+TH-10, and were run using the above conditions with a 55°C annealing temperature. All reactions were pooled and the 2.9 kbp product was purified by Qiaex extraction from an agarose gel as described above.

Additional sequences determined for TcbA<sub>ii</sub> internal peptides are disclosed herein as SEQ ID NO:21 and SEQ ID NO:22. As before, degenerate oligonucleotides (Reverse primers TH-17 and TH-18) were made corresponding to the reverse complement of sequences that encode a portion of the amino acid sequence of these peptides.

Table 14  
From SEQ ID NO:21

Amino Acid	Met	Glu	Thr	Gln	Asn	Ile	Gln	Glu	Pro
TH-17	3'-TAC	CTT/C	TGI	GTT/C	TTA/G	TAI	GTT/C	GTT/C	GG-5'

Table 15  
From SEQ ID NO:22

Amino Acid	Asn	Pro	Ile	Asn	Ile	Asn	Thr	Gly	Ile	Asp
TH-18	3'-TT(A/G)	GGI	TAI	TT(A/G)	TAI	TT(A?G)	TGI	CCI	TAI	CT(A/G)-5'

Degenerate oligonucleotides TH-18 and TH-17 were used in an amplification experiment with *Photobacterium luminescens* W-14 DNA as template and primers TH-4, TH-5, TH-6, or TH-7 as the 5'-(Forward) primers. These reactions amplified products of approximately 4 kbp and 4.5 kbp, respectively. These DNAs were transferred from agarose gels to nylon membranes and hybridized with a <sup>32</sup>P-labeled probe (as described above) prepared from the 2.9 kbp product amplified by the TH-5+TH10 primer pair. Both the 4 kbp and the 4.5 kbp amplification products hybridized strongly to the 2.9 kbp probe. These results were used to construct a map ordering the TcbA<sub>ii</sub> internal peptide sequences as shown in Fig. 3. Approximate distances between the primers are shown in nucleotides in Fig. 3.

#### 15 DNA Sequence of the 2.9 kbp TcbA<sub>ii</sub>-encoding fragment

Approximately 200 ng of the purified 2.9 kbp fragment (prepared above) was precipitated with ethanol and dissolved in 17 ml of water. One-half of this was used as sequencing template with 25 pmol of the TH-5 pool as primers, the other half was used as template for TH-10 priming. Sequencing reactions were as given in Example 8. No reliable sequence was produced using the TH-10 primer pool; however, reactions with TH-5 primer pool produced the sequence disclosed below:.

```

25  1  AATCGTGTG ATCCCTATGC CGNGCCGGT TCGGTGGAAT CGATGTCCTC ACCGGGGGTT
    61  TATTNGAGGG ANTNGTCCCG TGAGGCCAAA AANTGGAATG AAAGAAGTTC AATTNTTAC
    121 CTAGATAAAC GTCGCCCGGN TTTAGAAAGN TTANTGNTCA GCCAGAAAAT TTTGGTTGAG
    181 GAAATTCCAC CGNTGGTTCT CTCTATTGAT TNGGGCCTGG CCGGGTTCGA ANNAAAACNA
    241 GGAAATNCAC AAGTTGAGGT GATGGNTTTG THGCNANCTT NTCGTTTAGG TGGGGAGAAA
    301 CCTTNTCANC ACGNTTNTGA AACTGTCCGG GAAATCGTCC ATGANCGTGA NCCAGGNTTN
30  361 CGCCATTGG

```

Based on this sequence, a sequencing primer (TH-21, 5'-CCGGGCGACGTTTATCTAGG-3') was designed to reverse complement bases 120-139, and initiate polymerization towards the 5' end (i.e., TH-5 end) of the gel-purified 2.9 kbp TcbA<sub>ii</sub>-encoding PCR fragment. The determined sequence is shown below, and is compared to the biochemically determined N-terminal peptide sequence of TcbA<sub>ii</sub> SEQ ID NO:1.

TcbA<sub>ii</sub> 2.9 kbp PCR fragment Sequence Confirmation

[Underlined amino acids = encoded by degenerate oligonucleotides]

5 SEQ ID NO:1 F I Q G Y S D L F G - - A  
 2.9 kbp seq GC ATG CAG GGG TAT AGT GAC CTG TTT GGT AAT CGT GCT  
                   M Q G Y S D L F G N R A

10 From the homology of the derived amino acid sequence to the biochemically determined one, it is clear that the 2.9 kbp PCR fragment represents the TcbA coding region. This 2.9 kbp fragment was then used as a hybridization probe to screen the *Photothabdus* W-14 genomic library prepared in Example 8 for cosmids containing the TcbA<sub>ii</sub>-encoding gene.

15

Screening the *Photothabdus* cosmid library

The 2.9 kb gel-purified PCR fragment was labeled with <sup>32</sup>P using the Boehringer Mannheim High Prime labeling kit as described in Example 8. Filters containing remnants of approximately 800 colonies from the cosmid library were screened as described previously (Example 8), and positive clones were streaked for isolated colonies and rescreened. Three clones (8A11, 25G8, and 26D1) gave positive results through several screening and characterization steps. No hybridization of the TcbA<sub>ii</sub>-specific probe was ever observed with any of the four cosmids identified in Example 8, and which contain the *tcaB* and *tcaC* genes. DNA from cosmids 8A11, 25G8, and 26D1 was digested with restriction enzymes Bgl 2, EcoR 1 or Hind 3 (either alone or in combination with one another), and the fragments were separated on an agarose gel and transferred to a nylon membrane as described in Example 8. The membrane was hybridized with <sup>32</sup>P-labeled probe prepared from the 4.5 kbp fragment (generated by amplification of *Photothabdus* genomic DNA with primers TH-5+TH-17). The patterns generated from cosmid DNAs 8A11 and 26D1 were identical to those generated with similarly-cut genomic DNA on the same membrane. It is concluded that cosmids 8A11 and 26D1 are accurate representations of the genomic TcbA<sub>ii</sub> encoding locus. However, cosmid 25G8 has a single Bgl 2 fragment which is slightly larger than the genomic DNA. This may result from positioning of the insert within the vector.

DNA sequence of the tcbA-encoding gene

The membrane hybridization analysis of cosmid 26D1 revealed that the 4.5 kbp probe hybridized to a single large EcoR 1 fragment (greater than 9 kbp). This fragment was gel purified and ligated into the EcoR 1 site of pBC KS (+) as described in Example 8, to generate plasmid pBC-S1/R1. The partial DNA sequence of the insert DNA of this plasmid was determined by "primer walking" from the flanking vector sequence, using procedures described in Example 8. Further sequence was generated by extension from new oligonucleotides designed from the previously determined sequence. When compared to the determined DNA sequence for the tcbA gene identified by other methods (disclosed herein as SEQ ID NO:11 as described in Example 12 below), complete homology was found to nucleotides 1-272, 319-826, 2578-3036, and 3068-3540 (total bases = 1712). It was concluded that both approaches can be used to identify DNA fragments encoding the TcbA<sub>ii</sub> peptide.

Analysis of the derived amino acid sequence of the tcbA gene.

The sequence of the DNA fragment identified as SEQ ID NO:11 encodes a protein whose derived amino acid sequence is disclosed herein as SEQ ID NO:12. Several features verify the identity of the gene as that encoding the TcbA<sub>ii</sub> protein. The TcbA<sub>ii</sub> N-terminal peptide (SEQ ID NO:1; Phe Ile Gln Gly Tyr Ser Asp Leu Phe Gly Asn Arg Ala) is encoded as amino acids 88-100. The TcbA<sub>ii</sub> internal peptide TcbA<sub>ii</sub>-PT81(a) (SEQ ID NO:23) is encoded as amino acids 1065-1077, and TcbA<sub>ii</sub>-PT81(b) (SEQ ID NO:24) is encoded as amino acids 1571-1592. Further, the internal peptide TcbA<sub>ii</sub>-PT56 (SEQ ID NO:22) is encoded as amino acids 1474-1488, and the internal peptide TcbA<sub>ii</sub>-PT103 (SEQ ID NO:24) is encoded as amino acids 1614-1639. It is obvious that this gene is an authentic clone encoding the TcbA<sub>ii</sub> peptide as isolated from insecticidal protein preparations of *Photorhabdus luminescens* strain W-14.

The protein isolated as peptide TcbA<sub>ii</sub> is derived from cleavage of a longer peptide. Evidence for this is provided by the fact that the nucleotides encoding the TcbA<sub>ii</sub> N-terminal peptide SEQ ID NO:1 are preceded by 261 bases (encoding 87 N-terminal-proximal amino acids) of a longer open reading frame (SEQ ID NO:11). This reading frame begins with nucleotides that encode the amino acid sequence Met Gln Asn Ser

Leu, which corresponds to the N-terminal sequence of the large peptide TcbA, and is disclosed herein as SEQ ID NO:16. It is thought that TcbA is the precursor protein for TcbA<sub>ii</sub>.

5 Relationship of tcbA, tcaB and tcaC genes.

The tcaB and tcaC genes are closely linked and may be transcribed as a single mRNA (Example 8). The tcbA gene is borne on cosmids that apparently do not overlap the ones harboring the tcaB and tcaC cluster, since the respective genomic library  
10 screens identified different cosmids. However, comparison of the amino sequences encoded by the tcaB and tcaC genes with the tcbA gene reveals a substantial degree of homology. The amino acid conservation (Protein Alignment Mode of MacVector™ Sequence  
15 Analysis Software, scoring matrix pam250, hash value = 2; Kodak Scientific Imaging Systems, Rochester, NY) is shown in Fig. 4. On the score line of each panel in Fig. 4, up carats (^) indicate homology or conservative amino acid changes, and down carats (v) indicate nonhomology.

This analysis shows that the amino acid sequence of the TcbA  
20 peptide from residues 1739 to 1894 is highly homologous to amino acids 441 to 603 of the TcaB<sub>i</sub> peptide (162 of the total 627 amino acids of P8; SEQ ID NO:28). In addition, the sequence of TcbA amino acids 1932 to 2459 is highly homologous to amino acids 12 to 531 of peptide TcaB<sub>ii</sub> (520 of the total 562 amino acids; SEQ  
25 ID NO:30). Considering that the TcbA peptide (SEQ ID NO:12) comprises 2505 amino acids, a total of 684 amino acids (27%) at the C-proximal end of it is homologous to the TcaB<sub>i</sub> or TcaB<sub>ii</sub> peptides, and the homologies are arranged colinear to the arrangement of the putative TcaB preprotein (SEQ ID NO:26). A  
30 sizeable gap in the TcbA homology coincides with the junction between the TcaB<sub>i</sub> and TcaB<sub>ii</sub> portions of the TcaB preprotein. Clearly the TcbA and TcaB gene products are evolutionarily related, and it is proposed that they share some common  
35 function(s) in *Photorhabdus*.

Example 10Characterization of zinc-metalloproteases in *Photorhabdus* Broth:  
Protease Inhibition, Classification, and Purification

5           Protease Inhibition and Classification Assays: Protease assays were performed using FITC-casein dissolved in water as substrate (0.08% final assay concentration). Proteolysis reactions were performed at 25°C for 1 h in the appropriate buffer with 25 µl of *Photorhabdus* broth (150 µl total reaction  
10 volume). Samples were also assayed in the presence and absence of dithiothreitol. After incubation, an equal volume of 12% trichloroacetic acid was added to precipitate undigested protein. Following precipitation for 0.5 h and subsequent centrifugation, 100 µl of the supernatant was placed into a 96-well microtiter  
15 plate and the pH of the solution was adjusted by addition of an equal volume of 4N NaOH. Proteolysis was then quantitated using a Fluoroskan II fluorometric plate reader at excitation and emission wavelengths of 485 and 538 nm, respectively. Protease activity was tested over a range from pH 5.0-10.0 in 0.5 units  
20 increments. The following buffers were used at 50 mM final concentration: sodium acetate (pH 5.0 - 6.5); Tris-HCL (pH 7.0 - 8.0); and bis-Tris propane (pH 8.5-10.0). To identify the class of protease(s) observed, crude broth was treated with a variety of protease inhibitors (0.5 µg/µl final concentration) and then  
25 examined for protease activity at pH 8.0 using the substrate described above. The protease inhibitors used included E-64 (L-trans-exopoxysaccinylleucylamido[4-, -guanidino]-butane), 3,4 dichloroisocoumarin, Leupeptin, pepstatin, amastatin, ethylenediaminetetraacetic acid (EDTA) and 1,10 phenanthroline.

30           Protease assays performed over a pH range revealed that indeed protease(s) were present which exhibited maximal activity at ~ pH 8.0 (Table 16). Addition of DTT did not have any effect on protease activity. Crude broth was then treated with a variety of protease inhibitors (Table 17). Treatment of crude  
35 broth with the inhibitors described above revealed that 1,10 phenanthroline caused complete inhibition of all protease activity when added at a final concentration of 50 µg, with the IC<sub>50</sub> = 5 µg in 100 µl of a 2 mg/ml crude broth solution. These data indicate that the most abundant protease(s) found in the

*Photorhabdus* broth are from the zinc-metalloprotease class of enzymes.

Table 16  
5 Effect of pH on the protease activity found in a Day 1 production of *Photorhabdus luminescens* (strain W-14).

	pH	Flu. Units <sup>a</sup> Activity <sup>b</sup>	Percent
10	5.0	3013 ± 78	17
	5.5	7994 ± 448	45
15	6.0	12965 ± 483	74
	6.5	14390 ± 1291	82
	7.0	14386 ± 1287	82
20	7.5	14135 ± 198	80
	8.0	17582 ± 831	100
25	8.5	16183 ± 953	92
	9.0	16795 ± 760	96
	9.5	16279 ± 1022	93
30	10.0	15225 ± 210	87

a Flu. Units = Fluorescence Units (Maximum = ~28,000; background = ~ 2200).

b. Percent activity relative to the maximum at pH 8.0

35

Table 17  
Effect of different protease inhibitors on the protease activity  
at pH 8 found in a Day 1 production of *Photorhabdus luminescens*  
(strain W-14).

Inhibitor	Corrected Flu. Units <sup>a</sup>	Percent Inhibition <sup>b</sup>
Control	13053	0
E-64	14259	0
1,10 Phenanthroline <sup>c</sup>	15	99
3,4 Dichloroisocoumarin <sup>d</sup>	7956	39
Leupeptin	13074	0
Pepstatin <sup>c</sup>	13441	0
Amastatin	12474	4
DMSO Control	12005	8
Methanol Control	12125	7

a. Corrected Flu. Units = Fluorescence Units - background(2200 flu. units).

b. Percent Inhibition relative to protease activity at pH 8.0.

c. Inhibitors were dissolved in methanol.

d. Inhibitors were dissolved in DMSO.

The isolation of a zinc-metalloprotease was performed by applying dialyzed 10-80% ammonium sulfate pellet to a Q Sepharose column equilibrated at 50 mM Na<sub>2</sub>PO<sub>4</sub>, pH 7.0 as described in Example 5 for *Photorhabdus* toxin. After extensive washing, a 0 to 0.5 M NaCl gradient was used to elute toxin protein. The majority of biological activity and protein was eluted from 0.15 - 0.45 M NaCl. However, it was observed that the majority of proteolytic activity was present in the 0.25-0.35 M NaCl fraction with some activity in the 0.15-0.25 M NaCl fraction. SDS PAGE analysis of the 0.25-0.35 M NaCl fraction showed a major peptide band of approximately 60 kDa. The 0.15-0.25 M NaCl fraction contained a similar 60 kDa band but at lower relative protein concentration. Subsequent gel filtration of this fraction using a Superose 12 HR 16/50 column resulted in a major peak migrating at 57.5 kDa that contained a predominant (> 90% of total stained protein) 58.5 kDa band by SDS PAGE analysis. Additional analysis of this fraction using various protease inhibitors as described above determined that the protease was a zinc-metalloprotease. Nearly all of the protease activity present in *Photorhabdus* broth at day 1 of fermentation corresponded to the ~58 kDa zinc-metalloprotease.

In yet a second isolation of zinc-metalloprotease(s), W-14 *Photorhabdus* broth grown for three days was taken and protease



activity was visualized using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) laced with gelatin as described in Schmidt, T.M., Bleakley, B. and Nealson, K.M. 1988. SDS running gels (5.5 x 8 cm) were made with 12.5 %  
5 polyacrylamide (40% stock solution of acrylamide/bis-acrylamide; Sigma Chemical Co., St. Louis, MO) into which 0.1% gelatin final concentration (Biorad EIA grade reagent; Richmond CA) was incorporated upon dissolving in water. SDS-stacking gels (1.0 x 8 cm) were made with 5% polyacrylamide, also laced with 0.1%  
10 gelatin. Typically, 2.5 µg of protein to be tested was diluted in 0.03 ml of SDS-PAGE loading buffer without dithiothreitol (DTT) and loaded onto the gel. Proteins were electrophoresed in SDS running buffer (Laemmli, U.K. 1970. Nature 227, 680) at 0° C and at 8 mA. After electrophoresis was complete, the gel was  
15 washed for 2 h in 2.5% (v/v) Triton X-100. Gels were then incubated for 1 h at 37 °C in 0.1 M glycine (pH 8.0). After incubation, gels were fixed and stained overnight with 0.1% amido black in methanol-acetic acid- water (30:10:60, vol./vol./vol.; Sigma Chemical Co.). Protease activity was visualized as light  
20 areas against a dark, amido black stained background due to proteolysis and subsequent diffusion of incorporated gelatin. At least three distinct bands produced by proteolytic activity at 58-, 41-, and 38 kDa were observed.

Activity assays of the different proteases in W-14 day three  
25 culture broth were performed using FITC-casein dissolved in water as substrate (0.02% final assay concentration). Proteolysis experiments were performed at 37 °C for 0-0.5 h in 0.1M Tris-HCl (pH 8.0) with different protein fractions in a total volume of 0.15 ml. Reactions were terminated by addition of an equal  
30 volume of 12% trichloroacetic acid (TCA) dissolved in water. After incubation at room temperature for 0.25 h, samples were centrifuged at 10,000 x g for 0.25 h and 0.10 ml aliquots were removed and placed into 96-well microtiter plates. The solution was then neutralized by the addition of an equal volume of 2 N  
35 sodium hydroxide, followed by quantitation using a Fluoroskan II fluorometric plate reader with excitation and emission wavelengths of 485 and 538 nm, respectively. Activity measurements were performed using FITC-Casein with different protease concentrations at 37° C for 0-10 min. A unit of

activity was arbitrarily defined as the amount of enzyme needed to produce 1000 fluorescent units/min and specific activity was defined as units/mg of protease.

Inhibition studies were performed using two zinc-metalloprotease inhibitors; 1,10 phenanthroline and N-( $\alpha$ -rhamnopyranosyloxyhydroxyphosphinyl)-Leu-Trp(phosphoramidon) with stock solutions of the inhibitors dissolved in 100% ethanol and water, respectively. Stock concentrations were typically 10 mg/ml and 5 mg/ml for 1,10 phenanthroline and phosphoramidon, respectively, with final concentrations of inhibitor at 0.5-1.0 mg/ml per reaction. Treatment of three day W-14 crude broth with 1,10 phenanthroline, an inhibitor of all zinc metalloproteases, resulted in complete elimination of all protease activity while treatment with phosphoramidon, an inhibitor of thermolysin-like proteases (Weaver, L.H., Kester, W.R., and Matthews, B.W. 1977. J. Mol. Biol. 114, 119-132), resulted in ~56% reduction of protease activity. The residual proteolytic activity could not be further reduced with additional phosphoramidon.

The proteases of three day W-14 *Photorhabdus* broth were purified as follows: 4.0 liters of broth were concentrated using an Amicon spiral ultra filtration cartridge Type S1Y100 attached to an Amicon M-12 filtration device. The flow-through material having native proteins less than 100 kDa in size (3.8 L) was concentrated to 0.375 L using an Amicon spiral ultra filtration cartridge Type S1Y10 attached to an Amicon M-12 filtration device. The retentate material contained proteins ranging in size from 10-100 kDa. This material was loaded onto a Pharmacia HR16/10 column which had been packed with PerSeptive Biosystem (Framington, MA) Poros® 50 HQ strong anion exchange packing that had been equilibrated in 10 mM sodium phosphate buffer (pH 7.0). Proteins were loaded on the column at a flow rate of 5 ml/min, followed by washing unbound protein with buffer until A<sub>280</sub> = 0.00. Afterwards, proteins were eluted using a NaCl gradient of 0-1.0 M NaCl in 40 min at a flow rate of 7.5 ml/min. Fractions were assayed for protease activity, *supra.*, and active fractions were pooled. Proteolytically active fractions were diluted with 50% (v/v) 10 mM sodium phosphate buffer (pH 7.0) and loaded onto a Pharmacia HR 10/10 Mono Q column equilibrated in 10 mM sodium phosphate. After washing the column with buffer until A<sub>280</sub> =

0.00, proteins were eluted using a NaCl gradient of 0-0.5 M NaCl for 1 h at a flow rate of 2.0 ml/min. Fractions were assayed for protease activity. Those fractions having the greatest amount of phosphoramidon-sensitive protease activity, the phosphoramidon sensitive activity being due to the 41/38 kDa protease, *infra.*, were pooled. These fractions were found to elute at a range of 0.15-0.25 M NaCl. Fractions containing a predominance of phosphoramidon-insensitive protease activity, the 58 kDa protease, were also pooled. These fractions were found to elute at a range of 0.25-0.35 M NaCl. The phosphoramidon-sensitive protease fractions were then concentrated to a final volume of 0.75 ml using a Millipore Ultrafree®-15 centrifugal filter device Biomax-5K NMWL membrane. This material was applied at a flow rate of 0.5 ml/min to a Pharmacia HR 10/30 column that had been packed with Pharmacia Sephadex G-50 equilibrated in 10 mM sodium phosphate buffer (pH 7.0)/ 0.1 M NaCl. Fractions having the maximal phosphoramidon-sensitive protease activity were then pooled and centrifuged over a Millipore Ultrafree®-15 centrifugal filter device Biomax-50K NMWL membrane. Proteolytic activity analysis, *supra.*, indicated this material to have only phosphoramidon-sensitive protease activity. Pooling of the phosphoramidon-insensitive protease, the 58 kDa protein, was followed by concentrating in a Millipore Ultrafree®-15 centrifugal filter device Biomax-50K NMWL membrane and further separation on a Pharmacia Superdex-75 column. Fractions containing the protease were pooled.

Analysis of purified 58- and 41/38 kDa purified proteases revealed that, while both types of protease were completely inhibited with 1,10 phenanthroline, only the 41/38 kDa protease was inhibited with phosphoramidon. Further analysis of crude broth indicated that protease activity of day 1 W-14 broth has 23% of the total protease activity due to the 41/38 kDa protease, increasing to 44% in day three W-14 broth.

Standard SDS-PAGE analysis for examining protein purity and obtaining amino terminal sequence was performed using 4-20% gradient MiniPlus SeptraGels purchased from Integrated Separation Systems (Natick, MA). Proteins to be amino-terminal sequenced were blotted onto PVDF membrane following purification, *infra.*, (ProBlott™ Membranes; Applied Biosystems, Foster City, CA).

visualized with 0.1% amido black, excised, and sent to Cambridge Prochem; Cambridge, MA, for sequencing.

Deduced amino terminal sequence of the 58- (SEQ ID NO:45) and 41/38 kDa (SEQ ID NO:44) proteases from three day old W-14 broth were DV-GSEKANEKLLK (SEQ ID NO: 45) and DSGDDDKVTNTDIHR (SEQ ID NO:44), respectively.

Sequencing of the 41/38 kDa protease revealed several amino termini, each one having an additional amino acid removed by proteolysis. Examination of the primary, secondary, tertiary and quaternary sequences for the 38 and 41 kDa polypeptides allowed for deduction of the sequence shown above and revealed that these two proteases are homologous.

#### Example 11, Part A

#### 15 Screening of *Photorhabdus* Genomic Library via use of Antibodies for Genes encoding TcbA Peptide

In parallel to the sequencing described above, suitable probing and sequencing was done based on the TcbA<sub>ii</sub> peptide (SEQ ID NO:1). This sequencing was performed by preparing bacterial culture broths and purifying the toxin as described in Examples 1 and 2 above.

Genomic DNA was isolated from the *Photorhabdus luminescens* strain W-14 grown in Grace's insect tissue culture medium. The bacteria were grown in 5 ml of culture medium in a 250 ml Erlenmeyer flask at 28°C and 250 rpm for approximately 24 hours. Bacterial cells from 100 ml of culture medium were pelleted at 5000 x g for 10 minutes. The supernatant was discarded, and the cell pellets then were used for the genomic DNA isolation.

The genomic DNA was isolated using a modification of the CTAB method described in Section 2.4.3 of Ausubel (*supra.*). The section entitled "Large Scale CsCl prep of bacterial genomic DNA" was followed through step 6. At this point, an additional chloroform/isoamyl alcohol (24:1) extraction was performed followed by a phenol/chloroform/isoamyl (25:24:1) extraction step and a final chloroform/isoamyl/alcohol (24:1) extraction. The DNA was precipitated by the addition of a 0.6 volume of isopropanol. The precipitated DNA was hooked and wound around the end of a bent glass rod, dipped briefly into 70% ethanol as a final wash, and dissolved in 3 ml of TE buffer.

The DNA concentration, estimated by optical density at 280/260 nm, was approximately 2 mg/ml.

Using this genomic DNA, a library was prepared.

Approximately 50 µg of genomic DNA was partly digested with Sau3  
5 Al. Then NaCl density gradient centrifugation was used to size  
fractionate the partially digested DNA fragments. Fractions  
containing DNA fragments with an average size of 12 kb, or  
larger, as determined by agarose gel electrophoresis, were  
ligated into the plasmid BluScript, Stratagene, La Jolla,  
10 California, and transformed into an *E. coli* DH5α or DHB10 strain.

Separately, purified aliquots of the protein were sent to  
the biotechnology hybridoma center at the University of  
Wisconsin, Madison for production of monoclonal antibodies to the  
proteins. The material that was sent was the HPLC purified  
15 fraction containing native bands 1 and 2 which had been denatured  
at 65°C, and 20 µg of which was injected into each of four mice.  
Stable monoclonal antibody-producing hybridoma cell lines were  
recovered after spleen cells from unimmunized mouse were fused  
with a stable myeloma cell line. Monoclonal antibodies were  
20 recovered from the hybridomas.

Separately, polyclonal antibodies were created by taking  
native agarose gel purified band 1 (see Example 1) protein which  
was then used to immunize a New Zealand white rabbit. The  
protein was prepared by excising the band from the native agarose  
25 gels, briefly heating the gel pieces to 65°C to melt the agarose,  
and immediately emulsifying with adjuvant. Freund's complete  
adjuvant was used for the primary immunizations and Freund's  
incomplete was used for 3 additional injections at monthly  
intervals. For each injection, approximately 0.2 ml of  
30 emulsified band 1, containing 50 to 100 micrograms of protein,  
was delivered by multiple subcutaneous injections into the back  
of the rabbit. Serum was obtained 10 days after the final  
injection and additional bleeds were performed at weekly  
intervals for 3 weeks. The serum complement was inactivated by  
35 heating to 56°C for 15 minutes and then stored at -20°C.

The monoclonal and polyclonal antibodies were then used to  
screen the genomic library for the expression of antigens which  
could be detected by the epitope. Positive clones were detected  
on nitrocellulose filter colony lifts. An immunoblot analysis of  
40 the positive clones was undertaken.

An analysis of the clones as defined by both immunoblot and Southern analysis resulted in the tentative identification of five classes of clones.

In the first class of clone was a gene encoding the peptide designated here as TcbA<sub>ii</sub>. Full DNA sequence of this gene (TcbA) was obtained. It is set forth as SEQ ID NO:11. Confirmation that the sequence encodes the internal sequence of SEQ ID NO:1 is demonstrated by the presence of SEQ ID NO:1 at amino acid number 88 from the deduced amino acid sequence created by the open reading frame of SEQ ID NO:11. This can be confirmed by referring to SEQ ID NO:12, which is the deduced amino acid sequence created by SEQ ID NO:11.

The second class of toxin peptides contains the segments referred to above as TcaB<sub>i</sub>, TcaB<sub>ii</sub> and TcaC. Following the screening of the library with the polyclonal antisera, this second class of toxin genes was identified by several clones which produced different size proteins, all of which cross-reacted with the polyclonal antibody on an immunoblot and were also found to share DNA homology on a Southern Blot. Sequence comparison revealed that they belonged to the gene complex designated TcaB and TcaC above.

Three other classes of antibody toxin clones were also isolated in the polyclonal screen. These classes produced proteins that cross-react with a polyclonal antibody and also shared DNA homology with the classes as determined by Southern blotting. The classes have been designated Class III, Class IV and Class V. It was also possible to identify monoclonals that cross-reacted with Class I, II, III, and IV. This suggests that all have regions of high protein homology. Thus, it appears that the *P. luminescens* extracellular protein genes represent a family of genes which are evolutionarily related.

To further pursue the concept that there might be evolutionarily related variations in the toxin peptides contained within this organism, two approaches have been undertaken to examine other strains of *P. luminescens* for the presence of related proteins. This was done both by PCR amplification of genomic DNA and by immunoblot analysis using the polyclonal and monoclonal antibodies.

The results indicate that related proteins are produced by *P. luminescens* strains WX-2, WX-3, WX-4, WX-5, WX-6, WX-7, WX-8, WX-11, WX-12, WX-15 and W-14.

5

Example 11, Part BSequence and analysis of Class III toxin clones - tcc

Further DNA sequencing was performed on plasmids isolated from Class III *E. coli* clones described in Example 11, Part A.

10 The nucleotide sequence was shown to be three closely linked open reading frames at this genomic locus. This locus was designated *tcc* with the three open reading frames designated *tccA* SEQ ID NO:56, *tccB* SEQ ID NO:58 and *tccC* SEQ ID NO:60 (Fig. 6B).

15 The deduced amino acid from the *tccA* open reading frame indicates the gene encodes a protein of 105,459 Da. This protein was designated TccA. The first 12 amino acids of this protein match the N-terminal sequence obtained from a 108 kDa protein, SEQ ID NO:7, previously identified as part of the toxin complex.

20 The deduced amino acid from the *tccB* open reading frame indicates this gene encodes a protein of 175,716 Da. This protein was designated TccB. The first 11 amino acids of this protein match the N-terminal sequence obtained from a protein with estimated molecular weight of 185 kDa, SEQ ID NO:8.

25 The deduced amino acid sequence of *tccC* indicated that this open reading frame encodes a protein of 111,694 Da and the protein product was designated TccC.

Example 12Characterization of *Photorhabdus* Strains

30

In order to establish that the collection described herein was comprised of *Photorhabdus* strains, the strains herein were assessed in terms of recognized microbiological traits that are characteristic of *Photorhabdus* and which differentiate it from  
35 other *Enterobacteriaceae* and *Xenorhabdus* spp. (Farmer, J.J. 1984. Bergey's Manual of Systemic Bacteriology, vol 1. pp. 510-511. (ed. Kreig N.R. and Holt, J.G.). Williams & Wilkins, Baltimore.; Akhurst and Boemare, 1988, Boemare et al., 1993). These characteristic traits are as follows: Gram's stain negative

rods, organism size of 0.5-2  $\mu\text{m}$  in width and 2-10  $\mu\text{m}$  in length, red/yellow colony pigmentation, presence of crystalline inclusion bodies, presence of catalase, inability to reduce nitrate, presence of bioluminescence, ability to take up dye from growth media, positive for protease production, growth-temperature range below 37°C, survival under anaerobic conditions and positively motile. (Table 18). Reference *Escherichia coli*, *Xenorhabdus* and *Photorhabdus* strains were included in all tests for comparison. The overall results are consistent with all strains being part of the family *Enterobacteriaceae* and the genus *Photorhabdus*.

A luminometer was used to establish the bioluminescence of each strain and provide a quantitative and relative measurement of light production. For measurement of relative light emitting units, the broths from each strain (cells and media) were measured at three time intervals after inoculation in liquid culture (6, 12, and 24 hr) and compared to background luminosity (uninoculated media and water). Prior to measuring light emission from the various broths, cell density was established by measuring light absorbance (560 nm) in a Gilford Systems (Oberlin, OH) spectrophotometer using a sipper cell. Appropriate dilutions were then made (to normalize optical density to 1.0 unit) before measuring luminosity. Aliquots of the diluted broths were then placed into cuvettes (300  $\mu\text{l}$  each) and read in a Bio-Orbit 1251 Luminometer (Bio-Orbit Oy, Twiku, Finland). The integration period for each sample was 45 seconds. The samples were continuously mixed (spun in baffled cuvettes) while being read to provide oxygen availability. A positive test was determined as being  $\geq 5$ -fold background luminescence ( $\sim 5$ -10 units). In addition, colony luminosity was detected with photographic film overlays and visually, after adaptation in a darkroom. The Gram's staining characteristics of each strain were established with a commercial Gram's stain kit (BBL, Cockeysville, MD) used in conjunction with Gram's stain control slides (Fisher Scientific, Pittsburgh, PA). Microscopic evaluation was then performed using a Zeiss microscope (Carl Zeiss, Germany) 100X oil immersion objective lens (with 10X ocular and 2X body magnification). Microscopic examination of individual strains for organism size, cellular description and inclusion bodies (the latter after logarithmic growth) was



performed using wet mount slides (10X ocular, 2X body and 40X objective magnification) with oil immersion and phase contrast microscopy with a micrometer (Akhurst, R.J. and Boemare, N.E. 1990. Entomopathogenic Nematodes in Biological Control (ed. Gaugler, R. and Kaya, H.). pp. 75-90. CRC Press, Boca Raton, USA.; Baghdiguian S., Boyer-Giglio M.H., Thaler, J.O., Bonnot G., Boemare N. 1993. Biol. Cell 79, 177-185.)). Colony pigmentation was observed after inoculation on Bacto nutrient agar, (Difco Laboratories, Detroit, MI) prepared as per label instructions.

10 Incubation occurred at 28°C and descriptions were produced after 5-7 days. To test for the presence of the enzyme catalase, a colony of the test organism was removed on a small plug from a nutrient agar plate and placed into the bottom of a glass test

15 added down the side of the tube. A positive reaction was recorded when bubbles of gas (presumptive oxygen) appeared immediately or within 5 seconds. Controls of uninoculated nutrient agar and hydrogen peroxide solution were also examined. To test for nitrate reduction, each culture was inoculated into

20 10 ml of Bacto Nitrate Broth (Difco Laboratories, Detroit, MI). After 24 hours incubation at 28°C, nitrite production was tested by the addition of two drops of sulfanilic acid reagent and two drops of alpha-naphthylamine reagent (see Difco Manual, 10th edition, Difco Laboratories, Detroit, MI, 1984). The generation

25 of a distinct pink or red color indicates the formation of nitrite from nitrate. The ability of each strain to uptake dye from growth media was tested with Bacto MacConkey agar containing the dye neutral red; Bacto Tergitol-7 agar containing the dye bromothymol blue and Bacto EMB Agar containing the dye eosin-Y

30 (agars from Difco Laboratories, Detroit, MI, all prepared according to label instructions). After inoculation on these media, dye uptake was recorded after incubation at 28°C for 5 days. Growth on these latter media is characteristic for members of the family *Enterobacteriaceae*. Motility of each strain was

35 tested using a solution of Bacto Motility Test Medium (Difco Laboratories, Detroit, MI) prepared as per label instructions. A butt-stab inoculation was performed with each strain and motility was judged macroscopically by a diffuse zone of growth spreading from the line of inoculum. In many cases, motility was also

observed microscopically from liquid culture under wet mount slides. Biochemical nutrient evaluation for each strain was performed using BBL Enterotube II (Benton, Dickinson, Germany). Product instructions were followed with the exception that

5 incubation was carried out at 28°C for 5 days. Results were consistent with previously cited reports for *Photorhabdus*. The production of protease was tested by observing hydrolysis of gelatin using Bacto gelatin (Difco Laboratories, Detroit, MI) plates made as per label instructions. Cultures were inoculated

10 and the plates were incubated at 28°C for 5 days. To assess growth at different temperatures, agar plates [2% proteose peptone #3 with two percent Bacto-Agar (Difco, Detroit, MI) in deionized water] were streaked from a common source of inoculum. Plates were sealed with Nesco® film and incubated at 20, 28 and

15 37°C for up to three weeks. Plates showing no growth at 37°C showed no cell viability after transfer to a 28°C incubator for one week. Oxygen requirements for *Photorhabdus* strains were tested in the following manner. A butt-stab inoculation into fluid thioglycolate broth medium (Difco, Detroit, MI) was made.

20 The tubes were incubated at room temperature for one week and cultures were then examined for type and extent of growth. The indicator resazurin demonstrates the level of medium oxidation or the aerobiosis zone (Difco Manual, 10th edition, Difco Laboratories, Detroit, MI). Growth zone results obtained for the

25 *Photorhabdus* strains tested were consistent with those of a facultative anaerobic microorganism.

Table 18

Taxonomic Traits of *Photorhabdus* Strains

30

## Traits Assessed\*

Strain	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
W-14	<u>-</u>	+	+	<u>rd</u> <u>S</u>	+	-	+	+	+	<u>O</u>	+	+	+	+	+	+	-
WX-1	-	+	+	<u>rd</u> <u>S</u>	+	-	+	+	+	<u>O</u>	+	+	+	+	+	+	-
WX-2	-	+	+	<u>rd</u> <u>S</u>	+	-	+	+	+	<u>O</u>	+	+	+	+	+	+	-
WX-3	-	+	+	<u>rd</u> <u>S</u>	+	-	+	+	+	<u>YT</u>	+	+	+	+	+	+	-
WX-4	-	+	+	<u>rd</u> <u>S</u>	+	-	+	+	+	<u>YT</u>	+	+	+	+	+	+	-
WX-5	-	+	+	<u>rd</u> <u>S</u>	+	-	+	+	+	<u>LO</u>	+	+	+	+	+	+	-

WX-6	-	+	+	rd s	+	-	+	+	+	LY	+	+	+	+	+	+
WX-7	-	+	+	rd s	+	-	+	+	+	R	+	+	+	+	+	+
WX-8	-	+	+	rd s	+	-	+	+	+	O	+	+	+	+	+	+
WX-9	-	+	+	rd s	+	-	+	+	+	YT	+	+	+	+	+	+
WX-10	-	+	+	rd s	+	-	+	+	+	Ro	+	+	+	+	+	+
WX-11	-	+	+	rd s	+	-	+	+	+	Ro	+	+	+	+	+	+
WX-12	-	+	+	rd s	+	-	+	+	+	O	+	+	+	+	+	+
WX-14	-	+	+	rd s	+	-	+	+	+	LR	+	+	+	+	+	+
WX-15	-	+	+	rd s	+	-	+	+	+	LR	+	+	+	+	+	+
H9	-	+	+	rd s	+	-	+	+	+	LY	+	+	+	+	+	+
Hb	-	+	+	rd s	+	-	+	+	+	YT	+	+	+	+	+	+
Hm	-	+	+	rd s	+	-	+	+	+	TY	+	+	+	+	+	+
HP88	-	+	+	rd s	+	-	+	+	+	LY	+	+	+	+	+	+
NC-1	-	+	+	rd s	+	-	+	+	+	O	+	+	+	+	+	+
W30	-	+	+	rd s	+	-	+	+	+	YT	+	+	+	+	+	+
WIR	-	+	+	rd s	+	-	+	+	+	RO	+	+	+	+	+	+
B2	-	+	+	rd s	+	-	+	+	+	R	+	+	+	+	+	+
43948	-	+	+	rd s	+	-	+	+	+	O	+	+	+	+	+	+
43949	-	+	+	rd s	+	-	+	+	+	O	+	+	+	+	+	+
43950	-	+	+	rd s	+	-	+	+	+	O	+	+	+	+	+	+
43951	-	+	+	rd s	+	-	+	+	+	O	+	+	+	+	+	+
43952	-	+	+	rd s	+	-	+	+	+	O	+	+	+	+	+	+

\* - A = Gram's stain, B=Crystalline inclusion bodies, C=Bioluminescence, D=Cell form, E=Motility, F=Nitrate reduction, G=Presence of catalase, H=Gelatin hydrolysis, I=Dye uptake, J=Pigmentation, K=Growth on EMB agar, L=Growth on MacConkey agar, M=Growth on Tergitol-7 agar, N=Facultative anaerobe, O=Growth at 20°C, P=Growth at 28°C, Q=Growth at 37°C, + - +/- = positive or negative for trait, rd=rod, S=sized within Genus descriptors, RO=red-orange, LR = light red, R= red, O= orange, Y= yellow, T= tan, LY= light yellow, YT= yellow tan, and LO= light orange.

10

Cellular fatty acid analysis is a recognized tool for bacterial characterization at the genus and species level (Tornabene, T.G. 1985. Lipid Analysis and the Relationship to

Chemotaxonomy in Methods in Microbiology, Vol 18, 203-244.;  
Goodfellow, M. and O'Donnell, A.G. 1993. Roots of Bacterial  
Systematics in Handbook of New Bacterial Systematics (ed.  
Goodfellow, M. & O'Donnell, A.G.) pp. 3-54. London: Academic  
5 Press Ltd.), these references are incorporated herein by  
reference, and were used to confirm that our collection was  
related at the genus level. Cultures were shipped to an  
external, contract laboratory for fatty acid methyl ester  
analysis (FAME) using a Microbial ID (MIDI, Newark, DE, USA)  
10 Microbial Identification System (MIS). The MIS system consists of  
a Hewlett Packard HP5890A gas chromatograph with a 25mm x 0.2mm  
5% methylphenyl silicone fused silica capillary column. Hydrogen  
is used as the carrier gas and a flame-ionization detector  
functions in conjunction with an automatic sampler, integrator  
15 and computer. The computer compares the sample fatty acid methyl  
esters to a microbial fatty acid library and against a  
calibration mix of known fatty acids. As selected by the  
contract laboratory, strains were grown for 24 hours at 28°C on  
trypticase soy agar prior to analysis. Extraction of samples was  
20 performed by the contract lab as per standard FAME methodology.  
There was no direct identification of the strains to any  
luminescent bacterial group other than *Photorhabdus*. When the  
cluster analysis was performed, which compares the fatty acid  
profiles of a group of isolates, the strain fatty acid profiles  
25 were related at the genus level.

The evolutionary diversity of the *Photorhabdus* strains in  
our collection was measured by analysis of PCR (Polymerase Chain  
Reaction) mediated genomic fingerprinting using genomic DNA from  
each strain. This technique is based on families of repetitive  
30 DNA sequences present throughout the genome of diverse bacterial  
species (reviewed by Versalovic, J., Schneider, M., DE Bruijn,  
F.J. and Lupski, J.R. 1994. *Methods Mol. Cell. Biol.*, 5, 25-40.).  
Three of these, repetitive extragenic palindromic sequence (REP),  
enterobacterial repetitive intergenic consensus (ERIC) and the  
35 BOX element are thought to play an important role in the  
organization of the bacterial genome. Genomic organization is  
believed to be shaped by selection and the differential  
dispersion of these elements within the genome of closely related  
bacterial strains can be used to discriminate these strains (e.g.

Louws, F.J., Fulbright, D.W., Stephens, C.T. and DE Bruijn, F.J. 1994. Appl. Environ. Micro. 60, 2286-2295.). Rep-PCR utilizes oligonucleotide primers complementary to these repetitive sequences to amplify the variably sized DNA fragments lying  
5 between them. The resulting products are separated by electrophoresis to establish the DNA "fingerprint" for each strain.

To isolate genomic DNA from our strains, cell pellets were resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) to a  
10 final volume of 10 ml and 12 ml of 5 M NaCl was then added. This mixture was centrifuged 20 min. at 15,000 x g. The resulting pellet was resuspended in 5.7 ml of TE and 300 µl of 10% SDS and 60 µl 20 mg/ml proteinase K (Gibco BRL Products, Grand Island, NY) were added. This mixture was incubated at 37 °C for 1 hr,  
15 approximately 10 mg of lysozyme was then added and the mixture was incubated for an additional 45 min. One milliliter of 5M NaCl and 800 µl of CTAB/NaCl solution (10% w/v CTAB, 0.7 M NaCl) were then added and the mixture was incubated 10 min. at 65°C, gently agitated, then incubated and agitated for an additional 20 min.  
20 to aid in clearing of the cellular material. An equal volume of chloroform/isoamyl alcohol solution (24:1, v/v) was added, mixed gently then centrifuged. Two extractions were then performed with an equal volume of phenol/chloroform/isoamyl alcohol (50:49:1). Genomic DNA was precipitated with 0.6 volume of isopropanol.  
25 Precipitated DNA was removed with a glass rod, washed twice with 70% ethanol, dried and dissolved in 2 ml of STE (10 mM Tris-HCl pH8.0, 10 mM NaCl, 1 mM EDTA). The DNA was then quantitated by optical density at 260 nm. To perform rep-PCR analysis of *Photorhabdus* genomic DNA the following primers were used, REP1R-  
30 1; 5'-IIIIICGICGICATCIGGC-3' and REP2-I; 5'-ICGICTTATCIGGCCTAC-3'. PCR was performed using the following 25µl reaction: 7.75 µl H<sub>2</sub>O, 2.5 µl 10X LA buffer (PanVera Corp., Madison, WI), 16 µl dNTP mix (2.5 mM each), 1 µl of each primer at 50 pM/µl, 1 µl DMSO, 1.5 µl genomic DNA (concentrations ranged from 0.075-0.480 µg/µl) and  
35 0.25 µl TaKaRa EX Taq (PanVera Corp., Madison, WI). The PCR amplification was performed in a Perkin Elmer DNA Thermal Cycler (Norwalk, CT) using the following conditions: 95°C/7 min. then 35 cycles of; 94°C/1 min., 44°C/1 min., 65°C/8 min., followed by 15 min. at 65°C. After cycling, the 25 µl reaction was added to 5 µl

of 5X gel loading buffer (0.25% bromophenol blue, 40% w/v sucrose in H<sub>2</sub>O). A 15x20cm 1%-agarose gel was then run in TBE buffer (0.09 M Tris-borate, 0.002 M EDTA) using 8 ul of each reaction. The gel was run for approximately 16 hours at 45v. Gels were then  
5 stained in 20 ug/ml ethidium bromide for 1 hour and destained in TBE buffer for approximately 3 hours. Polaroid® photographs of the gels were then taken under UV illumination.

The presence or absence of bands at specific sizes for each strain was scored from the photographs and entered as a  
10 similarity matrix in the numerical taxonomy software program, NTSYS-pc (Exeter Software, Setauket, NY). Controls of *E. coli* strain HB101 and *Xanthomonas oryzae* pv. *oryzae* assayed at the same time produced PCR "fingerprints" corresponding to published reports (Versalovic, J., Koeuth, T. and Lupski, J.R. 1991.  
15 Nucleic Acids Res. 19, 6823-6831; Vera Cruz, C.M., Halda-Alija, L., Louws, F., Skinner, D.Z., George, M.L., Nelson, R.J., DE Bruijn, F.J., Rice, C. and Leach, J.E. 1995. Int. Rice Res. Notes, 20, 23-24.; Vera Cruz, C.M., Ardales, E.Y., Skinner, D.Z., Talag, J., Nelson, R.J., Louws, F.J., Leung, H., Mew, T.W. and  
20 Leach, J.E. 1996. Phytopathology (in press, respectively). The data from *Photorhabdus* strains were then analyzed with a series of programs within NTSYS-pc; SIMQUAL (Similarity for Qualitative data) to generate a matrix of similarity coefficients (using the Jaccard coefficient) and SAHN (Sequential, Agglomerative,  
25 Heirarchical and Nested) clustering [using the UPGMA (Unweighted Pair-Group Method with Arithmetic Averages) method] which groups related strains and can be expressed as a phenogram (Figure 5). The COPH (cophenetic values) and MXCOMP (matrix comparison) programs were used to generate a cophenetic value matrix and  
30 compare the correlation between this and the original matrix upon which the clustering was based. A resulting normalized Mantel statistic (r) was generated which is a measure of the goodness of fit for a cluster analysis (r=0.8-0.9 represents a very good fit). In our case r = 0.919. Therefore, our collection is  
35 comprised of a diverse group of easily distinguishable strains representative of the *Photorhabdus* genus.

Example 13  
Insecticidal Utility of Toxin(s) Produced  
by Various *Photorhabdus* Strains

5 Initial "seed" cultures of the various *Photorhabdus* strains were produced by inoculating 175 ml of 2% Proteose Peptone #3 (PP3) (Difco Laboratories, Detroit, MI) liquid media with a primary variant subclone in a 500 ml tribaffled flask with a Delong neck, covered with a Kaput. Inoculum for each seed culture  
10 was derived from oil-overlay agar slant cultures or plate cultures. After inoculation, these flasks were incubated for 16 hrs at 28°C on a rotary shaker at 150 rpm. These seed cultures were then used as uniform inoculum sources for a given fermentation of each strain. Additionally, overlaying the post-  
15 log seed culture with sterile mineral oil, adding a sterile magnetic stir bar for future resuspension and storing the culture in the dark, at room temperature provided long-term preservation of inoculum in a toxin-competent state. The production broths were inoculated by adding 1% of the actively growing seed culture  
20 to fresh 2% PP3 media (e.g. 1.75 ml per 175 ml fresh media). Production of broths occurred in either 500 ml tribaffled flasks (see above), or 2800 ml baffled, convex bottom flasks (500 ml volume) covered by a silicon foam closure. Production flasks were incubated for 24-48 hrs under the above mentioned  
25 conditions. Following incubation, the broths were dispensed into sterile 1 L polyethylene bottles, spun at 2600 x g for 1 hr at 10°C and decanted from the cell and debris pellet. The liquid broth was then vacuum filtered through Whatman GF/D (2.7 µm retention) and GF/B (1.0 µm retention) glass filters to remove  
30 debris. Further broth clarification was achieved with a tangential flow microfiltration device (Pall Filtron, Northborough, MA) using a 0.5 µm open-channel filter. When necessary, additional clarification could be obtained by chilling the broth (to 4°C) and centrifuging for several hours at 2600 x  
35 g. Following these procedures, the broth was filter sterilized using a 0.2 µm nitrocellulose membrane filter. Sterile broths were then used directly for biological assay, biochemical analysis or concentrated (up to 15-fold) using a 10,000 MW cut-off, M12 ultra-filtration device (Amicon, Beverly MA) or

centrifugal concentrators (Millipore, Bedford, MA and Pall Filtron, Northborough, MA) with a 10,000 MW pore size. In the case of centrifugal concentrators, the broth was spun at 2000 x g for approximately 2 hr. The 10,000 MW permeate was added to the  
5 corresponding retentate to achieve the desired concentration of components greater than 10,000 MW. Heat inactivation of processed broth samples was achieved by heating the samples at 100°C in a sand-filled heat block for 10 minutes.

The broth(s) and toxin complex(es) from different  
10 *Photorhabdus* strains are useful for reducing populations of insects and were used in a method of inhibiting an insect population which comprises applying to a locus of the insect an effective insect inactivating amount of the active described. A demonstration of the breadth of insecticidal activity observed  
15 from broths of a selected group of *Photorhabdus* strains fermented as described above is shown in Table 19. It is possible that additional insecticidal activities could be detected with these strains through increased concentration of the broth or by employing different fermentation methods. Consistent with the  
20 activity being associated with a protein, the insecticidal activity of all strains tested was heat labile (see above).

Culture broth(s) from diverse *Photorhabdus* strains show differential insecticidal activity (mortality and/or growth inhibition, reduced adult emergence) against a number of insects.  
25 More specifically, the activity is seen against corn rootworm larvae and boll weevil larvae which are members of the insect order Coleoptera. Other members of the Coleoptera include wireworms, pollen beetles, flea beetles, seed beetles and Colorado potato beetle. Activity is also observed against aster  
30 leafhopper and corn plant hopper, which are members of the order Homoptera. Other members of the Homoptera include planthoppers, pear psylla, apple sucker, scale insects, whiteflies, spittle bugs as well as numerous host specific aphid species. The broths and purified toxin complex(es) are also active against tobacco  
35 budworm, tobacco hornworm and European corn borer which are members of the order Lepidoptera. Other typical members of this order are beet armyworm, cabbage looper, black cutworm, corn earworm, codling moth, clothes moth, Indian mealmoth, leaf rollers, cabbage worm, cotton bollworm, bagworm, Eastern tent



caterpillar, sod webworm and fall armyworm. Activity is also seen against fruitfly and mosquito larvae which are members of the order *Diptera*. Other members of the order *Diptera* are, pea midge, carrot fly, cabbage root fly, turnip root fly, onion fly, crane fly and house fly and various mosquito species. Activity with broth(s) and toxin complex(es) is also seen against two-spotted spider mite which is a member of the order *Acarina* which includes strawberry spider mites, broad mites, citrus red mite, European red mite, pear rust mite and tomato russet mite.

Activity against corn rootworm larvae was tested as follows. *Photorhabdus* culture broth(s) (0-15 fold concentrated, filter sterilized), 2% Proteose Peptone #3, purified toxin complex(es) [0.23 mg/ml] or 10 mM sodium phosphate buffer, pH 7.0 were applied directly to the surface (about 1.5 cm<sup>2</sup>) of artificial diet (Rose, R. I. and McCabe, J. M. (1973). *J. Econ. Entomol.* 66, (398-400) in 40 µl aliquots. Toxin complex was diluted in 10 mM sodium phosphate buffer, pH 7.0. The diet plates were allowed to air-dry in a sterile flow-hood and the wells were infested with single, neonate *Diabrotica undecimpunctata howardi* (Southern corn rootworm, SCR) hatched from surface sterilized eggs. The plates were sealed, placed in a humidified growth chamber and maintained at 27°C for the appropriate period (3-5 days). Mortality and larval weight determinations were then scored. Generally, 16 insects per treatment were used in all studies. Control mortality was generally less than 5%.

Activity against boll weevil (*Anthonomus grandis*) was tested as follows. Concentrated (1-10 fold) *Photorhabdus* broths, control medium (2% Proteose Peptone #3), purified toxin complex(es) [0.23 mg/ml] or 10 mM sodium phosphate buffer, pH 7.0 were applied in 60 µl aliquots to the surface of 0.35 g of artificial diet (Stoneville Yellow lepidopteran diet) and allowed to dry. A single, 12-24 hr boll weevil larva was placed on the diet, and the wells were sealed and held at 25°C, 50% RH for 5 days. Mortality and larval weights were then assessed. Control mortality ranged between 0-13%.

Activity against mosquito larvae was tested as follows. The assay was conducted in a 96-well microtiter plate. Each well contained 200 µl of aqueous solution (10-fold concentrated *Photorhabdus* culture broth(s), control medium (2% Proteose

Peptone #3), 10 mM sodium phosphate buffer, toxin complex(es) (0.23 mg/ml or H<sub>2</sub>O) and approximately 20, 1-day old larvae (*Aedes aegypti*). There were 6 wells per treatment. The results were read at 3-4 days after infestation. Control mortality was  
5 between 0-20%.

Activity against fruitflies was tested as follows. Purchased *Drosophila melanogaster* medium was prepared using 50% dry medium and a 50% liquid of either water, control medium (2% Proteose Peptone #3), 10-fold concentrated *Photorhabdus* culture  
10 broth(s), purified toxin complex(es) [0.23 mg/ml] or 10 mM sodium phosphate buffer, pH 7.0. This was accomplished by placing 4.0 ml of dry medium in each of 3 rearing vials per treatment and adding 4.0 ml of the appropriate liquid. Ten late instar  
15 *Drosophila melanogaster* maggots were then added to each 25 ml vial. The vials were held on a laboratory bench, at room temperature, under fluorescent ceiling lights. Pupal or adult counts were made after 15 days of exposure. Adult emergence as compared to water and control medium (0-16% reduction).

Activity against aster leafhopper adults (*Macrostelus severini*) and corn planthopper nymphs (*Peregrinus maidis*) was tested with an ingestion assay designed to allow ingestion of the active without other external contact. The reservoir for the active/"food" solution is made by making 2 holes in the center of the bottom portion of a 35X10 mm Petri dish. A 2 inch Parafilm  
25 M<sup>®</sup> square is placed across the top of the dish and secured with an "O" ring. A 1 oz. plastic cup is then infested with approximately 7 hoppers and the reservoir is placed on top of the cup, Parafilm down. The test solution is then added to the reservoir through the holes. In tests using 10-fold concentrated  
30 *Photorhabdus* culture broth(s), the broth and control medium (2% Proteose Peptone #3) were dialyzed against 10 mM sodium phosphate buffer, pH 7.0 and sucrose (to 5%) was added to the resulting solution to reduce control mortality. Purified toxin complex(es) [0.23 mg/ml] or 10 mM sodium phosphate buffer, pH 7.0 was also  
35 tested. Mortality is reported at day 3. The assay was held in an incubator at 28°C, 70% RH with a 16/8 photoperiod. The assays were graded for mortality at 72 hours. Control mortality was less than 6%.

Activity against lepidopteran larvae was tested as follows. Concentrated (10-fold) *Photorhabdus* culture broth(s), control medium (2% Proteose Peptone #3), purified toxin complex(es) [0.23 mg/ml] or 10 mM sodium phosphate buffer, pH 7.0 were applied directly to the surface (~1.5 cm<sup>2</sup>) of standard artificial lepidopteran diet (Stoneville Yellow diet) in 40 µl aliquots. The diet plates were allowed to air-dry in a sterile flow-hood and each well was infested with a single, neonate larva. European corn borer (*Ostrinia nubilalis*) and tobacco hornworm (*Manduca sexta*) eggs were obtained from commercial sources and hatched in-house, whereas tobacco budworm (*Heliothis virescens*) larvae were supplied internally. Following infestation with larvae, the diet plates were sealed, placed in a humidified growth chamber and maintained in the dark at 27°C for the appropriate period. Mortality and weight determinations were scored at day 5. Generally, 16 insects per treatment were used in all studies. Control mortality generally ranged from 4-12.5% for control medium and was less than 10% for phosphate buffer.

Activity against two-spotted spider mite (*Tetranychus urticae*) was determined as follows. Young squash plants were trimmed to a single cotyledon and sprayed to run-off with 10-fold concentrated broth(s), control medium (2% Proteose Peptone #3), purified toxin complex(es) [0.23 mg/ml] or 10 mM sodium phosphate buffer, pH 7.0. After drying, the plants were infested with a mixed population of spider mites and held at lab temperature and humidity for 72 hr. Live mites were then counted to determine levels of control.

Table 19  
Observed Insecticidal Spectrum of Broths From Different  
*Photorhabdus* Strains

	<i>Photorhabdus</i> Strain	Sensitive* Insect Species
5	WX-1	3**, 4, 5, 6, 7, 8
	WX-2	2, 4
	WX-3	1, 4
	WX-4	1, 4
10	WX-5	4
	WX-6	4
	WX-7	3, 4, 5, 6, 7, 8
	WX-8	1, 2, 4
	WX-9	1, 2, 4
15	WX-10	4
	WX-11	1, 2, 4
	WX-12	2, 4, 5, 6, 7, 8
	WX-14	1, 2, 4
	WX-15	1, 2, 4
20	W30	3, 4, 5, 8
	NC-1	1, 2, 3, 4, 5, 6, 7, 8, 9
	WIR	2, 3, 5, 6, 7, 8
	HP88	1, 3, 4, 5, 7, 8
	Hb	3, 4, 5, 7, 8
25	Hm	1, 2, 3, 4, 5, 7, 8
	H9	1, 2, 3, 4, 5, 6, 7, 8
	W-14	1, 2, 3, 4, 5, 6, 7, 8, 10
	ATCC 43948	4
	ATCC 43949	4
30	ATCC 43950	4
	ATCC 43951	4
	ATCC 43952	4

\* =  $\geq 25\%$  mortality and/or growth inhibition vs. control

35 \*\* = 1; Tobacco budworm, 2; European corn borer, 3;  
Tobacco hornworm, 4; Southern corn rootworm, 5;  
Boll weevil, 6; Mosquito, 7; Fruit Fly, 8;  
Aster Leafhopper, 9; Corn planthopper, 10;  
Two-spotted spider mite.

Example 14Non W-14 Photorhabdus Strains:Purification, Characterization and Activity Spectrum5    Purification

The protocol, as follows, is similar to that developed for the purification of W-14 and was established based on purifying those fractions having the most activity against Southern corn root worm (SCR), as determined in bioassays (see Example 13).

10   Typically, 4-20 L of broth that had been filtered, as described in Example 13, were received and concentrated using an Amicon spiral ultra filtration cartridge Type SLV100 attached to an Amicon M-12 filtration device. The retentate contained native proteins consisting of molecular sizes greater than 100 kDa,

15   whereas the flow through material contained native proteins less than 100 kDa in size. The majority of the activity against SCR was contained in the 100 kDa retentate. The retentate was then continually diafiltered with 10 mM sodium phosphate (pH = 7.0) until the filtrate reached an  $A_{280} < 0.100$ . Unless otherwise

20   stated, all procedures from this point were performed in buffer as defined by 10 mM sodium phosphate (pH 7.0). The retentate was then concentrated to a final volume of approximately 0.20 L and filtered using a 0.45 mm Nalgene™ Filterware sterile filtration unit. The filtered material was loaded at 7.5 ml/min onto a

25   Pharmacia HR16/10 column which had been packed with PerSeptive Biosystem Poros® 50 HQ strong anion exchange matrix equilibrated in buffer using a PerSeptive Biosystem Sprint® HPLC system. After loading, the column was washed with buffer until an  $A_{280} < 0.100$  was achieved. Proteins were then eluted from the column at

30   2.5 ml/min using buffer with 0.4 M NaCl for 20 min for a total volume of 50 ml. The column was then washed using buffer with 1.0 M NaCl at the same flow rate for an additional 20 min (final volume = 50 ml). Proteins eluted with 0.4 M and 1.0 M NaCl were placed in separate dialysis bags (Spectra/Por® Membrane MWCO:

35   2,000) and allowed to dialyze overnight at 4° C in 12 L buffer. The majority of the activity against SCR was contained in the 0.4 M fraction. The 0.4 M fraction was further purified by application of 20 ml to a Pharmacia XK 26/100 column that had been prepacked with Sepharose CL4B (Pharmacia) using a flow rate

of 0.75 ml/min. Fractions were pooled based on A280 peak profile and concentrated to a final volume of 0.75 ml using a Millipore Ultrafree®-15 centrifugal filter device Biomax-50K NMWL membrane. Protein concentrations were determined using a Biorad Protein Assay Kit with bovine gamma globulin as a standard.

#### Characterization

The native molecular weight of the SCR toxin complex was determined using a Pharmacia HR 16/50 that had been prepacked with Sepharose CL4B in buffer. The column was then calibrated using proteins of known molecular size thereby allowing for calculation of the toxin approximate native molecular size. As shown in Table 20, the molecular size of the toxin complex ranged from 777 kDa with strain Hb to 1,900 kDa with strain WX-14. The yield of toxin complex also varied, from strain WX-12 producing 0.8 mg/L to strain Hb, which produced 7.0 mg/L.

Proteins found in the toxin complex were examined for individual polypeptide size using SDS-PAGE analysis. Typically, 20 mg protein of the toxin complex from each strain was loaded onto a 2-15% polyacrylamide gel (Integrated Separation Systems) and electrophoresed at 20 mA in Biorad SDS-PAGE buffer. After completion of electrophoresis, the gels were stained overnight in Biorad Coomassie blue R-250 (0.2% in methanol: acetic acid: water; 40:10:40 v/v/v). Subsequently, gels were destained in methanol:acetic acid: water; 40:10:40 (v/v/v). The gels were then rinsed with water for 15 min and scanned using a Molecular Dynamics Personal Laser Densitometer®. Lanes were quantitated and molecular sizes were calculated as compared to Biorad high molecular weight standards, which ranged from 200-45 kDa.

Sizes of the individual polypeptides comprising the SCR toxin complex from each strain are listed in Table 21. The sizes of the individual polypeptides ranged from 230 kDa with strain WX-1 to a size of 16 kDa, as seen with strain WX-7. Every strain, with the exception of strain Hb, had polypeptides comprising the toxin complex that were in the 160-230 kDa range, the 100-160 kDa range, and the 50-80 kDa range. These data indicate that the toxin complex may vary in peptide composition and components from strain to strain, however, in all cases the

toxin attributes appears to consist of a large, oligomeric protein complex.

Table 20  
Characterization of a Toxin Complex From  
Non W-14 *Photorhabdus* Strains

Strain	Approx. Native Molecular Wt. <sup>a</sup>	Yield Active Fraction (mg/L) <sup>b</sup>
H9	972,000	1.8
Hb	777,000	7.0
Hm	1,400,000	1.1
HP88	813,000	2.5
NC1	1,092,000	3.3
WIR	979,000	1.0
WX-1	973,000	0.8
WX-2	951,000	2.2
WX-7	1,000,000	1.5
WX-12	898,000	0.4
WX-14	1,900,000	1.9
W-14	860,000	7.5

a Native molecular weight determined using a Pharmacia HR 16/50 column packed with Sepharose CL4B

b Amount of toxin complex recovered from culture broth.

### Activity Spectrum

As shown in Table 21, the toxin complexes purified from strains Hm and H9 were tested for activity against a variety of insects, with the toxin complex from strain W-14 for comparison. The assays were performed as described in Example 13. The toxin complex from all three strains exhibited activity against tobacco bud worm, European corn borer, Southern corn root worm, and aster leafhopper. Furthermore, the toxin complex from strains Hm and W-14 also exhibited activity against two-spotted spider mite. In addition, the toxin complex from W-14 exhibited activity against mosquito larvae. These data indicate that the toxin complex, while having similarities in activities between certain orders of insects, can also exhibit differential activities against other orders of insects.

Table 21

The Approximate Sizes (in kDa) of Peptides in a Purified  
Toxin Complex From Non W-14 *Photorhabdus*

5

H9	Hb	Hm	HP 88	NC-1	WIR	WX-1	WX-2	WX-7	WX-12	WX-14	W-11
180	150	170	170	180	170	230	200	200	180	210	190
170	140	140	160	170	160	190	170	180	160	180	180
160	139	100	140	140	120	170	150	110	140	160	170
140	130	81	130	110	110	160	120	87	139	120	160
120	120	72	129	44	89	110	110	75	130	110	150
98	100	68	110	16	79	98	82	43	110	100	130
87	98	49	100		74	76	64	33	92	95	120
84	88	46	86		62	58	37	28	87	80	110
79	81	30	81		51	53	30	26	80	69	93
72	75	22	77		40	41		23	73	49	90
68	69	20	73		39	35		22	59	41	77
60	60	19	60		37	31		21	56	33	69
57	57		58		33	28		19	51		65
52	54		45		30	24		18	37		63
46	49		39		28	22		16	33		60
40	44		35		27				32		51
37	39				25				26		46
	37				23						40
	35										39
											29



Table 22

Observed Insecticidal Spectrum of a Purified Toxin Complex from  
*Photorhabdus* Strains

5	<u><i>Photorhabdus</i> Strain</u>	<u>Sensitive* Insect Species</u>
	Hm Toxin Complex	1**, 2, 3, 5, 6, 7, 8
	H9 Toxin Complex	1, 2, 3, 6, 7, 8
10	W-14 Toxin Complex	1, 2, 3, 4, 5, 6, 7, 8
	* = > 25% mortality or growth inhibition	
	* = > 25% mortality or growth inhibition	
15	** = 1; Tobacco bud worm, 2; European corn borer, 3; Southern corn root worm, 4; Mosquito, 5; Two-spotted spider mite, 6; Aster Leafhopper, 7; Fruit Fly, 8; Boll Weevil	

Example 15Sub-Fractionation of *Photorhabdus* Protein Toxin Complex

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The *Photorhabdus* protein toxin complex was isolated as described in Example 14. Next, about 10 mg toxin was applied to a MonoQ 5/5 column equilibrated with 20 mM Tris-HCl, pH 7.0 at a flow rate of 1ml/min. The column was washed with 20 mM Tris-HCl, pH 7.0 until the optical density at 280 nm returned to baseline absorbance. The proteins bound to the column were eluted with a linear gradient of 0 to 1.0 M NaCl in 20 mM Tris-HCl, pH 7.0 at 1 ml/min for 30 min. One ml fractions were collected and subjected to Southern corn rootworm (SCR) bioassay (see Example 13). Peaks of activity were determined by a series of dilutions of each fraction in SCR bioassays. Two activity peaks against SCR were observed and were named A (eluted at about 0.2-0.3 M NaCl) and B (eluted at 0.3-0.4 M NaCl). Activity peaks A and B were pooled separately and both peaks were further purified using a 3-step procedure described below.

35

Solid  $(\text{NH}_4)_2\text{SO}_4$  was added to the above protein fraction to a final concentration of 1.7 M. Proteins were then applied to a phenyl-Superose 5/5 column equilibrated with 1.7 M  $(\text{NH}_4)_2\text{SO}_4$  in 50 mM potassium phosphate buffer, pH 7 at 1 ml/min. Proteins bound to the column were eluted with a linear gradient of 1.7 M  $(\text{NH}_4)_2\text{SO}_4$ , 0% ethylene glycol, 50 mM potassium phosphate, pH 7.0 to 25% ethylene glycol, 25 mM potassium phosphate, pH 7.0 (no  $(\text{NH}_4)_2\text{SO}_4$ ) at 0.5 ml/min. Fractions were dialyzed overnight

40

against 10 mM sodium phosphate buffer, pH 7.0. Activities in each fraction against SCR were determined by bioassay.

The fractions with the highest activity were pooled and applied to a MonoQ 5/5 column which was equilibrated with 20 mM Tris-HCl, pH 7.0 at 1 ml/min. The proteins bound to the column were eluted at 1 ml/min by a linear gradient of 0 to 1M NaCl in 20 mM Tris-HCl, pH 7.0.

For the final step of purification, the most active fractions above (determined by SCR bioassay) were pooled and subjected to a second phenyl-Superose 5/5/ column. Solid  $(\text{NH}_4)_2\text{SO}_4$  was added to a final concentration of 1.7 M. The solution was then loaded onto the column equilibrated with 1.7 M  $(\text{NH}_4)_2\text{SO}_4$  in 50 mM potassium phosphate buffer, pH 7 at 1ml/min. Proteins bound to the column were eluted with a linear gradient of 1.7 M  $(\text{NH}_4)_2\text{SO}_4$ , 50 mM potassium phosphate, pH 7.0 to 10 mM potassium phosphate, pH 7.0 at 0.5 ml/min. Fractions were dialyzed overnight against 10 mM sodium phosphate buffer, pH 7.0. Activities in each fraction against SCR were determined by bioassay.

The final purified protein by the above 3-step procedure from peak A was named toxin A and the final purified protein from peak B was named toxin B.

#### Characterization and Amino Acid Sequencing of Toxin A and Toxin B

In SDS-PAGE, both toxin A and toxin B contained two major (> 90% of total Commassie stained protein) peptides: 192 kDa (named A1 and B1, respectively) and 58 kDa (named A2 and B2, respectively). Both toxin A and toxin B revealed only one major band in native PAGE, indicating A1 and A2 were subunits of one protein complex, and B1 and B2 were subunits of one protein complex. Further, the native molecular weight of both toxin A and toxin B were determined to be 860 kDa by gel filtration chromatography. The relative molar concentrations of A1 to A2 was judged to be a 1 to 1 equivalence as determined by densitometric analysis of SDS-PAGE gels. Similarly, B1 and B2 peptides were present at the same molar concentration.

Toxin A and toxin B were electrophoresed in 10% SDS-PAGE and transblotted to PVDF membranes. Blots were sent for amino acid analysis and N-terminal amino acid sequencing at Harvard MicroChem and Cambridge ProChem, respectively. The N-terminal

amino sequence of B1 was determined to be identical to SEQ ID NO:1, the TcbA<sub>ii</sub> region of the *tcbA* gene (SEQ ID NO:12, position 87 to 99). A unique N-terminal sequence was obtained for peptide B2 (SEQ ID NO:40). The N-terminal amino acid sequence of peptide  
5 B2 was identical to the TcbA<sub>iii</sub> region of the derived amino acid sequence for the *tcbA* gene (SEQ ID NO:12, position 1935 to 1945). Therefore, the B toxin contained predominantly two peptides, TcbA<sub>ii</sub> and TcbA<sub>iii</sub>, that were observed to be derived from the same gene product, TcbA.

10 The N-terminal sequence of A2 (SEQ ID NO:41) was unique in comparison to the TcbA<sub>iii</sub> peptide and other peptides. The A2 peptide was denoted TcdA<sub>iii</sub> (see Example 17). SEQ ID NO:6 was determined to be a mixture of amino acid sequences SEQ ID NO:40 and 41.

15 Peptides A1 and A2 were further subjected to internal amino acid sequencing. For internal amino acid sequencing, 10 µg of toxin A was electrophoresized in 10% SDS-PAGE and transblotted to PVDF membrane. After the blot was stained with amido black, peptides A1 and A2, denoted TcdA<sub>ii</sub> and TcdA<sub>iii</sub>, respectively,  
20 were excised from the blot and sent to Harvard MicroChem and Cambridge ProChem. Peptides were subjected to trypsin digestion followed by HPLC chromatography to separate individual peptides. N-terminal amino acid analysis was performed on selected tryptic peptide fragments. Two internal amino acid sequences of peptide  
25 A1 (TcdA<sub>ii</sub>-PK71, SEQ ID NO:38 and TcdA<sub>ii</sub>-PK44, SEQ ID NO:39) were found to have significant homologies with deduced amino acid sequences of the TcbA<sub>ii</sub> region of the *tcbA* gene (SEQ ID NO:12). Similarly, the N-terminal sequence (SEQ ID NO:41) and two  
internal sequences of peptides A2 (TcdA<sub>iii</sub>-PK57, SEQ ID NO:42 and  
30 TcdA<sub>iii</sub>-PK20, SEQ ID NO:43) also showed significant homology with deduced amino acid sequences of TcbA<sub>iii</sub> region of the *tcbA* gene (SEQ ID NO:12).

In summary of above results, the toxin complex has at least two active protein toxin complexes against SCR; toxin A and toxin  
35 B. Toxin A and toxin B are similar in their native and subunits molecular weight, however, their peptide compositions are different. Toxin A contained peptides TcdA<sub>ii</sub> and TcdA<sub>iii</sub> as the major peptides and the toxin B contains TcbA<sub>ii</sub> and TcbA<sub>iii</sub> as the major peptides.

Example 16Cleavage and Activation of TcbA Peptide

5

In the toxin B complex, peptide TcbA<sub>ii</sub> and TcbA<sub>iii</sub> originate from the single gene product TcbA (Example 15). The processing of TcbA peptide to TcbA<sub>ii</sub> and TcbA<sub>iii</sub> is presumably by the action of *Photorhabdus* protease(s), and most likely, the metalloproteases described in Example 10. In some cases, it was noted that when *Photorhabdus* W-14 broth was processed, TcbA peptide was present in toxin B complex as a major component, in addition to peptides TcbA<sub>ii</sub> and TcbA<sub>iii</sub>. Identical procedures, described for the purification of toxin B complex (Example 15), were used to enrich peptide TcbA from toxin complex fraction of W-14 broth. The final purified material was analyzed in a 4-20% gradient SDS-PAGE and major peptides were quantified by densitometry. It was determined that TcbA, TcbA<sub>ii</sub> and TcbA<sub>iii</sub> comprised 58%, 36%, and 6%, respectively, of total protein. The identities of these peptides were confirmed by their respective molecular sizes in SDS-PAGE and Western blot analysis using monospecific antibodies. The native molecular weight of this fraction was determined to be 860 kDa.

The cleavage of TcbA was evaluated by treating the above purified material with purified 38 kDa and 58 kDa W-14 *Photorhabdus* metalloproteases (Example 10), and Trypsin as a control enzyme (Sigma, MO). The standard reaction consisted 17.5  $\mu$ g the above purified fraction, 1.5 unit protease, and 0.1 M Tris buffer, pH 8.0 in a total volume of 100  $\mu$ l. For the control reaction, protease was omitted. The reaction mixtures were incubated at 37 °C for 90 min. At the end of the reaction, 20  $\mu$ l was taken and boiled with SDS-PAGE sample buffer immediately for electrophoresis analysis in a 4-20% gradient SDS-PAGE. It was determined from SDS-PAGE that in both 38 kDa and 58 kDa protease treatments, the amount of peptides TcbA<sub>ii</sub> and TcbA<sub>iii</sub> increased about 3-fold while the amount of TcbA peptide decreased proportionally (Table 23). The relative reduction and augmentation of selected peptides was confirmed by Western blot analyses. Furthermore, gel filtration of the cleaved material revealed that the native molecular size of the complex remained the same. Upon trypsin treatment, peptides TcbA and TcbA<sub>ii</sub> were

nonspecifically digested into small peptides. This indicated that 38 kDa and 58 kDa *Photorhabdus* proteases can specifically process peptide TcbA into peptides TcbA<sub>ii</sub> and TcbA<sub>iii</sub>. Protease treated and untreated control of the remaining 80 ul reaction mixture were serial diluted with 10 mM sodium phosphate buffer, pH 7.0 and analyzed by SCR bioassay. By comparing activity in several dilution, it was determined that the 38 kDa protease treatment increased SCR insecticidal activity approximately 3 to 4 fold. The growth inhibition of remaining insects in the protease treatment was also more severe than control (Table 23).

Table 23

Conversion and activation of peptide TcbA into peptides TcbA<sub>ii</sub> and TcbA<sub>iii</sub> by protease treatment.

	Control	38 kDa protease treatment
S0 (% of total protein)	58	18
S1 (% of total protein)	36	64
S9 (% of total protein)	6	18
LD50 (ug protein)	2.1	0.52
SCR Weight (mg/insect)*	0.2	0.1

\*: an indication of growth inhibition by measuring the average weight of live insect after 5 days on diet in the assay.

25

Example 17Screening of the library for a gene encoding the TcdA<sub>ii</sub> Peptide

The cloning and characterization of a gene encoding the TcdA<sub>ii</sub> peptide, described as SEQ ID NO:17 (internal peptide TcdA<sub>ii</sub>-PT111 N-terminal sequence) and SEQ ID NO:18 (internal peptide TcdA<sub>ii</sub>-PT79 N-terminal sequence) was completed. Two pools of degenerate oligonucleotides, designed to encode the amino acid sequences of SEQ ID NO:17 (Table 24) and SEQ ID NO:18 (Table 25), and the reverse complements of those sequences, were synthesized as described in Example 8. The DNA sequence of the oligonucleotides is given below:

Table 24  
Degenerate Oligonucleotide for SEQ ID NO:17

P2-PT111	1	2	3	4	5	6	7	8
Amino Acid	Ala	Phe	Asn	Ile	Asp	Asp	Val	Ser
Codons	5' GCN	TT(T/C)	AA(T/C)	AT(T/C/A)	GA(T/C)	GA(T/C)	GTN 3'	
P2.3.6.CB	5' GC(A/C/G/T)	TT(T/C)	AAT	ATT	GAT	GAT	GT 3'	
P2.3.5	5' GC(A/C/G/T)	TT(T/C)	AA(T/C)	AT(T/C/A)	GA(T/C)	GA(T/C)	GT 3'	
P2.3.5R	5' AC	(G/A)TC	(G/A)TC	(T/G/A)AT	(G/A)TT	(G/A)AA	(A/C/G/T)GC 3'	
P2.3.5RI	5' ACI	TCI	TCI	ATI	TTI	AAI	GC 3'	
P2.3R.CB	5' CAG	(A/G)CT	(A/C)AC	ATC	ATC	AAT	ATT	AAA 3'

Table 25  
Degenerate Oligonucleotide for SEQ ID NO:18

P2-PT79	1	2	3	4	5	6	7	8	9	10	11	12	13
Amino Acid	Phe	Ile	Val	Tyr	Thr	Ser	Leu	Gly	Val	Asn	Pro	Asn	Asn
Codons*	5' TTY	ATH	GTN	TAY	ACN	6	6	GGN	GTN	AAV	CCN	AAV	AAV 3'
P2.79.2	5' TTY	ATY	GTK	TAT	ACY	TCI	YTR	GGY	GTK	AAT	CCR	AAT	AAT 3'
P2.79.3	5' TTT	ATT	GTK	TAT	ACY	AGY	YTR	GGY	GTK	AAT	CCR	AAT	AAT 3'
P2.79.R.1	5' ATT	ATT	YGG	ATT	MAC	RCC	YAR	RCT	RGT	ATA	MAC	AAT	AAA 3'
P2.79R.CB	5' ATT	ATT	YGG	ATT	MAC	ACC	CAG	RCT	GGT	ATA	MAC	AAT	AAA 3'

\* According to IUPAC-IUB codes for nucleotides, Y = C or T, H = A, C or T,  
N = A, C, G or T, K = G or T, R = A or G, and M = A or C

Polymerase Chain Reactions (PCR) were performed essentially as described in Example 8, using as forward primers P2.3.6.CB or P2.3.5, and as reverse primers P2.79.R.1 or P2.79R.CB, in all forward/reverse combinations, using *Photorhabdus* W-14 genomic DNA as template. In another set of reactions, primers P2.79.2 or P2.79.3 were used as forward primers, and P2.3.5R, P2.3.5RI, and P2.3R.CB were used as reverse primers in all forward/reverse combinations. Only in the reactions containing P2.3.6.CB as the forward primers combined with P2.79.R.1 or P2.79R.CB as the reverse primers was a non-artifactual amplified product seen, of estimated size (mobility on agarose gels) of 2500 base pairs. The order of the primers used to obtain this amplification product indicates that the peptide fragment TcdA<sub>ii</sub>-PT111 lies amino-proximal to the peptide fragment TcdA<sub>ii</sub>-PT79.

The 2500 bp PCR products were ligated to the plasmid vector pCR™II (Invitrogen, San Diego, CA) according to the supplier's instructions, and the DNA sequences across the ends of the insert fragments of two isolates (HS24 and HS27) were determined using the supplier's recommended primers and the sequencing methods described previously. The sequence of both isolates was the same. New primers were synthesized based on the determined sequence, and used to prime additional sequencing reactions to obtain a total of 2557 bases of the insert [SEQ ID NO:36]. Translation of the partial peptide encoded by SEQ ID No: 36 yields the 845 amino acid sequence disclosed as SEQ ID NO:37. Protein homology analysis of this portion of the TcdA<sub>ii</sub> peptide fragment reveals substantial amino acid homology (68% similarity; 53% identity) to residues 542 to 1390 of protein TcbA [SEQ ID NO:12]. It is therefore apparent that the gene represented in part by SEQ ID NO:36 produces a protein of similar, but not identical, amino acid sequence as the TcbA protein, and which likely has similar, but not identical biological activity as the TcbA protein.

In yet another instance, a gene encoding the peptides TcdA<sub>ii</sub>-PK44 and the TcdA<sub>ii</sub> 58 kDa N-terminal peptide, described as SEQ ID NO:9 (internal peptide TcdA<sub>ii</sub>-PK44 sequence), and SEQ ID NO:41 (TcdA<sub>ii</sub> 58 kDa N-terminal peptide sequence) was isolated. Two pools of degenerate oligonucleotides, designed to encode the amino acid sequences described as SEQ ID NO:39 (Table 27) and SEQ

ID NO:41 (Table 26), and the reverse complements of those sequences, were synthesized as described in Example 3. and their DNA sequences.



Table 26  
Degenerate Oligonucleotide for SEQ ID NO:41

Codon #	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Amino Acid	Leu	Arg	Ser	Ala	Asn	Thr	Leu	Thr	Asp	Leu	Phe	Leu	Pro	Gln
A2.1	5' YTR	CGY	AGY	GCI	ANT	ACY	YTR	ACY	GAT	YTR	TTT	YTR	OCR	CA 3'
A2.2				GCI	ANT	ACI	YTR	ACI	GAY	YTR	TTY	YTR	OCI	CA 3'
A2.3.R		5' TG	YCG	YAR	AAA	YAR	RTC	RGT	YAR	RGT	RTT	IGC	RCT	RCG 3'
A2.4.R				5' TG	ICG	CAG	AAA	CAG	RTC	IGT	CAG	IGT	ATT	IGC 3'

Table 27  
Degenerate Oligonucleotide for SEQ ID NO:39

Amino Acid #	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
Codon #	1	2	3	4	5	6	7	8	9
Amino Acid	Gly	Pro	Val	Glu	Ile	Asn	Thr	Ala	Ile
A1.44.1	5' GGY	CCR	GTK	GAA	ATT	AAT	ACC	GCI	AT 3'
A1.44.1R	5' ATI	GCG	GTA	TTA	ATT	TCM	ACY	GGR	CC 3'
A1.44.2	5' GGI	CCI	GTI	GAR	ATY	AAY	ACI	GCI	AT 3'
A1.44.2R	5' ATI	GCI	GTR	TTR	ATY	TCI	ACI	GCI	CC 3'

Polymerase Chain Reactions (PCR) were performed essentially as described in Example 8, using as forward primers A1.44.1 or A1.44.2, and reverse primers A2.3R or A2.4R, in all forward/reverse combinations, using *Photothabdus* W-14 genomic DNA as template. In another set of reactions, primers A2.1 or A2.2 were used as forward primers, and A1.44.1R, and A1.44.2R were used as reverse primers in all forward/reverse combinations. Only in the reactions containing A1.44.1 or A1.44.2 as the forward primers combined with A2.3R as the reverse primer was a non-artifactual amplified product seen, of estimated size (mobility on agarose gels) of 1400 base pairs. The order of the primers used to obtain this amplification product indicates that the peptide fragment TcdA<sub>iii</sub>-PK44 lies amino-proximal to the 58 kDa peptide fragment of TcdA<sub>iii</sub>.

The 1400 bp PCR products were ligated to the plasmid vector pCR™II according to the supplier's instructions. The DNA sequences across the ends of the insert fragments of four isolates were determined using primers similar in sequence to the supplier's recommended primers and using sequencing methods described previously. The nucleic acid sequence of all isolates differed as expected in the regions corresponding to the degenerate primer sequences, but the amino acid sequences deduced from these data were the same as the actual amino acid sequences for the peptides determined previously, (SEQ ID NOS:41 and 39).

Screening of the W-14 genomic cosmid library as described in Example 8 with a radiolabeled probe comprised of the DNA prepared above (SEQ ID NO:36) identified five hybridizing cosmid isolates, namely 17D9, 20B10, 21D2, 27B10, and 26D1. These cosmids were distinct from those previously identified with probes corresponding to the genes described as SEQ ID NO:11 or SEQ ID NO:25. Restriction enzyme analysis and DNA blot hybridizations identified three EcoR I fragments, of approximate sizes 3.7, 3.7, and 1.1 kbp, that span the region comprising the DNA of SEQ ID NO:36. Screening of the W-14 genomic cosmid library using as probe the radiolabeled 1.4 kbp DNA fragment prepared in this example identified the same five cosmids (17D9, 20B10, 21D2, 27B10, and 26D1). DNA blot hybridization to EcoR I-digested cosmid DNAs also showed hybridization to the same subset

of EcoR I fragments as seen with the 2.5 kbp TcdA<sub>ii</sub> gene probe, indicating that both fragments are encoded on the genomic DNA.

DNA sequence determination of the cloned EcoR I fragments revealed an uninterrupted reading frame of 7551 base pairs (SEQ ID NO:46), encoding a 282.9 kDa protein of 2516 amino acids (SEQ ID NO:47). Analysis of the amino acid sequence of this protein revealed all expected internal fragments of peptides TcdA<sub>ii</sub> (SEQ ID NOS:17, 18, 37, 38 and 39) and the TcdA<sub>iii</sub> peptide N-terminus (SEQ ID NO:41) and all TcdA<sub>iii</sub> internal peptides (SEQ ID NOS:42 and 43). The peptides isolated and identified as TcdA<sub>ii</sub> and TcdA<sub>iii</sub> are each products of the open reading frame, denoted *tcdA*, disclosed as SEQ ID NO:46. Further, SEQ ID NO:47 shows, starting at position 89, the sequence disclosed as SEQ ID NO:13, which is the N-terminal sequence of a peptide of size approximately 201 kDa, indicating that the initial protein produced from SEQ ID No: 46 is processed in a manner similar to that previously disclosed for SEQ ID NO:12. In addition, the protein is further cleaved to generate a product of size 209.2 kDa, encoded by SEQ ID NO:48 and disclosed as SEQ ID NO:49 (TcdA<sub>ii</sub> peptide), and a product of size 63.6 kDa, encoded by SEQ ID NO:50 and disclosed as SEQ ID NO:51 (TcdA<sub>iii</sub> peptide). Thus, it is thought that the insecticidal activity identified as toxin A (Example 15) derived from the products of SEQ ID NO:46, as exemplified by the full-length protein of 282.9 kDa disclosed as SEQ ID NO:47, is processed to produce the peptides disclosed as SEQ ID NOS:49 and 51. It is thought that the insecticidal activity identified as toxin B (Example 15) derives from the products of SEQ ID NO:11, as exemplified by the 280.6 kDa protein disclosed as SEQ ID NO:12. This protein is proteolytically processed to yield the 207.6 kDa peptide disclosed as SEQ ID NO:53, which is encoded by SEQ ID NO:52, and the 62.9 kDa peptide having N-terminal sequence disclosed as SEQ ID NO:40, and further disclosed as SEQ ID NO:55, which is encoded by SEQ ID NO:54.

Amino acid sequence comparisons between the proteins disclosed as SEQ ID NO:12 and SEQ ID NO:47 reveal that they have 69% similarity and 54% identity. This high degree of evolutionary relationship is not uniform throughout the entire amino acid sequence of these peptides, but is higher towards the carboxy-terminal end of the proteins, since the peptides

disclosed as SEQ ID NO:51 (derived from SEQ ID NO:47) and SEQ ID NO:55 (derived from SEQ ID NO:12) have 76% similarity and 64% identity.

5

Example 18

Control of European Cornborer-Induced Leaf Damage on Maize Plants  
by Spray Application of *Photorhabdus* (Strain W-14) Broth

10       The ability of *Photorhabdus* toxin(s) to reduce plant damage  
caused by insect larvae was demonstrated by measuring leaf damage  
caused by European corn borer (*Ostrinia nubilalis*) infested onto  
maize plants treated with *Photorhabdus* broth. Fermentation broth  
from *Photorhabdus* strain W-14 was produced and concentrated  
15 approximately 10-fold using ultrafiltration (10,000 MW pore-size)  
as described in Example 13. The resulting concentrated broth was  
then filter sterilized using 0.2 micron nitrocellulose membrane  
filters. A similarly prepared sample of uninoculated 2% proteose  
peptone #3 was used for control purposes. Maize plants (a  
20 DowElanco proprietary inbred line) were grown from seed to  
vegetative stage 7 or 8 in pots containing a soilless mixture in  
a greenhouse (27°C day; 22°C night, about 50%RH, 14 hr day-  
length, watered/fertilized as needed). The test plants were  
arranged in a randomized complete block design (3 reps/treatment,  
25 6 plants/treatment) in a greenhouse with temperature about 22°C  
day; 18°C night, no artificial light and with partial shading,  
about 50%RH and watered/fertilized as needed. Treatments  
(uninoculated media and concentrated *Photorhabdus* broth) were  
applied with a syringe sprayer, 2.0 mls applied from directly  
30 (about 6 inches) over the whorl and 2.0 additional mls applied in  
a circular motion from approximately one foot above the whorl.  
In addition, one group of plants received no treatment. After  
the treatments had dried (approximately 30 minutes), twelve  
neonate European corn borer larvae (eggs obtained from commercial  
35 sources and hatched in-house) were applied directly to the whorl.  
After one week, the plants were scored for damage to the leaves  
using a modified Guthrie Scale (Koziel, M. G., Beland, G. L.,  
Bowman, C., Carozzi, N. B., Crenshaw, R., Crossland, L., Dawson,  
J., Desai, N., Hill, M., Kadwell, S., Launis, K., Lewis, K.,  
40 Maddox, D., McPherson, K., Meghji, M. Z., Merlin, E., Rhodes, R.,

Warren, G. W., Wright, M. and Evola, S. V. 1993).

Bio/Technology, 11, 194-195.) and the scores were compared statistically [T-test (LSD)  $p < 0.05$  and Tukey's Studentized Range (HSD) Test  $p < 0.1$ ]. The results are shown in Table 28. For reference, a score of 1 represents no damage, a score of 2 represents fine "window pane" damage on the unfurled leaf with no pinhole penetration and a score of 5 represents leaf penetration with elongated lesions and/or mid rib feeding evident on more than three leaves (lesions  $< 1$  inch). These data indicate that broth or other protein containing fractions may confer protection against specific insect pests when delivered in a sprayable formulation or when the gene or derivative thereof, encoding the protein or part thereof, is delivered via a transgenic plant or microbe.

Table 28

Effect of *Photorhabdus* Culture Broth on  
European Corn Borer-Induced Leaf Damage on Maize

Treatment	Average Guthrie Score
No Treatment	5.02 <sup>a</sup>
Uninoculated medium	5.15 <sup>a</sup>
<i>Photorhabdus</i> Broth	2.24 <sup>b</sup>
Means with different letters are statistically different ( $p < 0.05$ or $p < 0.1$ ).	

#### Example 19

#### Genetic Engineering of Genes for Expression in *E. coli*

#### Summary of constructions

A series of plasmids were constructed to express the *tcba* gene of *Photorhabdus* W-14 in *Escherichia coli*. A list of the plasmids is shown in Table 29. A brief description of each construction follows as well as a summary of the *E. coli* expression data obtained.

Table 29  
Expression plasmids for the *tcba* gene.

Plasmid	Gene	Vector/Selection	Compartment
pDAB634	<i>tcba</i>	pBC/Chl	Intracellular
pAcGP67B/ <i>tcba</i>	<i>tcba</i>	pAcGP67B/Amp	Baculovirus, secreted
pDAB635	<i>tcba</i>	pET27b/Kan	Periplasm
pET15- <i>tcba</i>	<i>tcba</i>	pET15- <i>tcba</i>	Intracellular

Abbreviations: Kan=kanamycin, Chl=chloramphenicol, Amp=ampicillin

5

#### Construction of pDAB634

In Example 9, a large EcoR I fragment which hybridizes to the *TcbA<sub>ij</sub>* probe is described. This fragment was subcloned into pBC (Stratagene, La Jolla CA). Sequence analysis indicates that this fragment is 8816 base pairs. The fragment encodes the *tcba* gene with the initiating ATG at position 571 and the terminating TAA at position 8086. The fragment therefore carries 570 base pairs of *Photorhabdus* DNA upstream of the ATG and 730 base pairs downstream of the TAA.

15

#### Construction of Plasmid pAcGP67B/*tcba*

The *tcba* gene was PCR amplified using the following primers; 5' primer (S1Ac51) 5' TTT AAA CCA TGG GAA ACT CAT TAT CAA GCA CTA TC 3' and 3' primer (S1Ac31) 5' TTT AAA GCG GCC GCT TAA CGG ATG GTA TAA CGA ATA TG 3'. PCR was performed using a TaKaRa LA PCR kit from PanVera (Madison, Wisconsin) in the following reaction: 57.5 ml water, 10 ml 10X LA buffer, 16 ml dNTPs (2.5 mM each stock solution), 20 ml each primer at 10 pmoles/ml, 300 ng of the plasmid pDAB634 containing the W-14 *tcba* gene and one ml of TaKaRa LA Taq polymerase. The cycling conditions were 98°C/20 sec, 68°C/5 min, 72°C/10 min for 30 cycles. A PCR product of the expected about 7526bp was isolated in a 0.8% agarose gel in TBE (100 mM Tris, 90 mM boric acid, 1 mM EDTA) buffer and purified using a Qiaex II kit from Qiagen (Chatsworth, California). The purified *tcba* gene was digested with Nco I and Not I and ligated into the baculovirus transfer vector pAcGP67B (PharMingen (San Diego, California)) and transformed into DH5 $\alpha$  *E. coli*. The *tcba* gene was then cut from pAcGP67B and transferred to pET27b to create plasmid pDAB635. A missense mutation in the *tcba* gene was repaired in pDAB635.

35

The repaired *tcbA* gene contains two changes from the sequence shown in Sequence ID NO:11; an A>G at 212 changing an asparagine 71 to serine 71 and a G>A at 229 changing an alanine 77 to threonine 77. These changes are both upstream of the proposed TcbA<sub>ii</sub> N-terminus.

#### Construction of pET15-*tcbA*

The *tcbA* coding region of pDAB635 was transferred to vector pET15b. This was accomplished using shotgun ligations, the DNAs were cut with restriction enzymes Nco I and Xho I. The resulting recombinant is called pET15-*tcbA*.

#### Expression of TcbA in *E. coli* from plasmid pET15-*tcbA*

Expression of *tcbA* in *E. coli* was obtained by modification of the methods previously described by Studier et al. (Studier, F.W., Rosenberg, A., Dunn, J., and Dubendorff, J., (1990) Use of T7 RNA polymerase to direct expression of cloned genes. Methods Enzymol., 185: 60-89.). Competent *E. coli* cells strain BL21(DE3) were transformed with plasmid pET15-*tcbA* and plated on LB agar containing 100 µg/ml ampicillin and 40 mM glucose. The transformed cells were plated to a density of several hundred isolated colonies/plate. Following overnight incubation at 37°C the cells were scraped from the plates and suspended in LB broth containing 100 µg /ml ampicillin. Typical culture volumes were from 200-500 ml. At time zero, culture densities (OD600) were from 0.05-0.15 depending on the experiment. Cultures were shaken at one of three temperatures (22°C, 30°C or 37°C) until a density of 0.15-0.5 was obtained at which time they were induced with 1 mM isopropylthio-β-galactoside (IPTG). Cultures were incubated at the designated temperature for 4-5 hours and then were transferred to 4°C until processing (12-72 hours).

#### Purification and characterization of TcbA expressed in *E.coli* from Plasmid pET15-*tcbA*.

*E. coli* cultures expressing TcbA peptides were processed as follows. Cells were harvested by centrifugation at 17,000 x G and the media was decanted and saved in a separate container.

The media was concentrated about 8x using the M12 (Amicon, Beverly MA) filtration system and a 100 kD molecular mass cut-off filter. The concentrated media was loaded onto an anion exchange

column and the bound proteins were eluted with 1.0 M NaCl. The 1.0 M NaCl elution peak was found to cause mortality against Southern corn rootworm (SCR) larvae (Table 30). The 1.0 M NaCl fraction was dialyzed against 10 mM sodium phosphate buffer pH 7.0, concentrated, and subjected to gel filtration on Sepharose CL-4B (Pharmacia, Piscataway, New Jersey). The region of the CL-4B elution profile corresponding to calculated molecular weight (about 900 kDa) as the native W-14 toxin complex was collected, concentrated and bioassayed against larvae. The collected 900 kDa fraction was found to have insecticidal activity (see Table 30 below), with symptomology similar to that caused by native W-14 toxin complex. This fraction was subjected to Proteinase K and heat treatment, the activity in both cases was either eliminated or reduced, providing evidence that the activity is proteinaceous in nature. In addition, the active fraction tested immunologically positive for the TcbA and TcbA<sub>iii</sub> peptides in immunoblot analysis when tested with an anti-TcbA<sub>iii</sub> monoclonal antibody (Table 30).

Table 30

Results of Immunoblot and SCR Bioassays.

Fraction	SCR Activity		Immunoblot Peptides Detected	Native Size [CL-4B Estimated Size]
	% Mortality	% Growth Inhibit.		
TcbA Media 1.0 M Ion Exchange	+++	+++	TcbA	
TcbA Media CL-4B	+++	+++	TcbA, TcbA <sub>iii</sub>	~900 kDa
TcbA Media CL-4B + Proteinase K	++	+++	NT	
TcbA Media CL-4B + heat treatment	-	-	NT	
TcbA Cell Sup CL-4B	-	+++	NT	~900 kD

PK = Proteinase K treatment 2 hours; Heat treatment = 100°C for 10 minutes; ND = None Detected; NT = Not Tested. Scoring system for mortality and growth inhibition as compared to control samples; 5-24%="+", 25-49%="++", 50-100%="+++".

The cell pellet was resuspended in 10 mM sodium phosphate buffer, pH=7.0, and lysed by passage through a Bio-Neb™ cell nebulizer (Glas-Col Inc., Terra Haute, IN). The pellets were



5 treated with DNase to remove DNA and centrifuged at 17,000 x g to  
separate the cell pellet from the cell supernatant. The  
supernatant fraction was decanted and filtered through a 0.2  
micron filter to remove large particles and subjected to anion  
exchange chromatography. Bound proteins were eluted with 1.0 M  
10 NaCl, dialyzed and concentrated using Biomax™ (Millipore Corp,  
Bedford, MA) concentrators with a molecular mass cut-off of  
50,000 Daltons. The concentrated fraction was subjected to gel  
filtration chromatography using Sepharose CL-4B beaded matrix.  
15 Bioassay data for material prepared in this way is shown in Table  
30 and is denoted as " TcbA Cell Sup".

In yet another method to handle large amounts of material,  
the cell pellets were re-suspended in 10 mM sodium phosphate  
buffer, pH = 7.0 and thoroughly homogenized by using a Kontes  
15 Glass Company (Vineland, NJ) 40 ml tissue grinder. The cellular  
debris was pelleted by centrifugation at 25,000 x g and the cell  
supernatant was decanted, passed through a 0.2 micron filter and  
subjected to anion exchange chromatography using a Pharmacia  
10/10 column packed with Poros HQ 50 beads. The bound proteins  
20 were eluted by performing a NaCl gradient of 0.0 to 1.0 M.  
Fractions containing the TcbA protein were combined and  
concentrated using a 50 kDa concentrator and subjected to gel  
filtration chromatography using Pharmacia CL-4B beaded matrix.  
The fractions containing TcbA oligomer, molecular mass of  
25 approximately 900 kDa, were collected and subjected to anion  
exchange chromatography using a Pharmacia Mono Q 10/10 column  
equilibrated with 20 mM Tris buffer pH = 7.3. A gradient of 0.0  
to 1.0 M NaCl was used to elute recombinant TcbA protein.  
Recombinant TcbA eluted from the column at a salt concentration  
30 of approximately 0.3-0.4 M NaCl, the same molarity at which  
native TcbA oligomer is eluted from the Mono Q 10/10 column. The  
recombinant TcbA fraction was found to cause SCR mortality in  
bioassay experiments similar to those in Table 30.

35

## SEQUENCE LISTING

- (1) GENERAL INFORMATION:
- 5 (i) APPLICANT: Ensign, Jerald C  
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- 20 (ii) TITLE OF INVENTION: Insecticidal Protein Toxins From  
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- (iii) NUMBER OF SEQUENCES: 61
- 25 (iv) CORRESPONDENCE ADDRESS:  
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35 (B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:  
40 (A) APPLICATION NUMBER:  
(B) FILING DATE:  
(C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:  
45 (A) APPLICATION NUMBER: US 08/063,615  
(B) FILING DATE: 18-MAY-1993
- (vii) PRIOR APPLICATION DATA:  
(A) APPLICATION NUMBER: US 08/395,497  
50 (B) FILING DATE: 28-FEB-1995
- (vii) PRIOR APPLICATION DATA:  
(A) APPLICATION NUMBER: US 60/007,255  
55 (B) FILING DATE: 06-NOV-1995
- (vii) PRIOR APPLICATION DATA:  
(A) APPLICATION NUMBER: US 08/608,423  
(B) FILING DATE: 23-FEB-1996

## (vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 08/705,484  
(B) FILING DATE: 23-AUG-1996

5

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15

## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: linear

20

## (ii) MOLECULE TYPE: protein

25

## (v) FRAGMENT TYPE: N-terminal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

30

Phe Ile Gln Gly Tyr Ser Asp Leu Phe Gly Asn  
1                      5                      10

35

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: linear

40

## (ii) MOLECULE TYPE: protein

45

## (v) FRAGMENT TYPE: N-terminal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

50

Met Gln Asp Ser Pro Glu Val Ser Ile Thr Thr Trp  
1                      5                      10

55

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: linear

60

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: N-terminal

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

10 Ser Glu Ser Leu Phe Thr Gln Thr Leu Lys Glu Ala Arg Arg Asp Ala  
1 5 10 15  
Leu Val Ala

15 (2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 14 amino acids  
(B) TYPE: amino acid  
20 (C) STRANDEDNESS:  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

30 Ala Ser Pro Leu Ser Thr Ser Glu Leu Thr Ser Lys Leu Asn  
1 5 10

35 (2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 9 amino acids  
(B) TYPE: amino acid  
40 (C) STRANDEDNESS:  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

45 (v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

50 Ala Gly Asp Thr Ala Asn Ile Gly Asp  
1 5

(2) INFORMATION FOR SEQ ID NO:6:

55 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 15 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
60 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: N-terminal

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Leu Gly Gly Ala Ala Thr Leu Leu Asp Leu Leu Leu Pro Gln Ile  
1 5 10 15

10

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 11 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

20

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

25

Met Leu Ser Thr Met Glu Lys Gln Leu Asn Glu  
1 5 10

30 (2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

35 (A) LENGTH: 9 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

40

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

45

Met Asn Leu Ala Ser Pro Leu Ile Ser  
1 5

50 (2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

55 (A) LENGTH: 16 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

60

(v) FRAGMENT TYPE: N-terminal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met Ile Asn Leu Asp Ile Asn Glu Gln Asn Lys Ile Met Val Val Ser  
 1 5 10 15

## (2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 20 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: N-terminal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Ala Ala Lys Asp Val Lys Phe Gly Ser Asp Ala Arg Val Lys Met Leu  
 1 5 10 15

Arg Gly Val Asn  
 20

## (2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 7515 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

## (ix) FEATURE:

(A) NAME/KEY: CDS  
 (B) LOCATION: 1..7515

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATG CAA AAC TCA TTA TCA AGC ACT ATC GAT ACT ATT TGT CAG AAA CTG 48  
 Met Gln Asn Ser Leu Ser Ser Thr Ile Asp Thr Ile Cys Gln Lys Leu  
 1 5 10 15

CAA TTA ACT TGT CCG GCG GAA ATT GCT TTG TAT CCC TTT GAT ACT TTC 96  
 Gln Leu Thr Cys Pro Ala Glu Ile Ala Leu Tyr Pro Phe Asp Thr Phe  
 20 25 30

CGG GAA AAA ACT CGG GGA ATG GTT AAT TGG GGG GAA GCA AAA CGG ATT 144  
 Arg Glu Lys Thr Arg Gly Met Val Asn Trp Gly Glu Ala Lys Arg Ile  
 35 40 45

TAT GAA ATT GCA CAA GCG GAA CAG GAT AGA AAC CTA CTT CAT GAA AAA 192  
 Tyr Glu Ile Ala Gln Ala Glu Gln Asp Arg Asn Leu Leu His Glu Lys  
 50 55 60

CGT ATT TTT GCC TAT GCT AAT CCG CTG CTG AAA AAC GCT GTT CGG TTG 240  
 Arg Ile Phe Ala Tyr Ala Asn Pro Leu Leu Lys Asn Ala Val Arg Leu

	65					70						75				80	
	GGT	ACC	CGG	CAA	ATG	TTG	GGT	TTT	ATA	CAA	GGT	TAT	AGT	GAT	CTG	TTT	283
5	Gly	Thr	Arg	Gln	Met	Leu	Gly	Phe	Ile	Gln	Gly	Tyr	Ser	Asp	Leu	Phe	
					85					90					95		
	GGT	AAT	CGT	GCT	GAT	AAC	TAT	GCC	GCG	CCG	GCC	TCG	GTT	GCA	TCG	ATG	336
10	Gly	Asn	Arg	Ala	Asp	Asn	Tyr	Ala	Ala	Pro	Gly	Ser	Val	Ala	Ser	Met	
				100					105					110			
	TTG	TCA	CCG	GCG	GCT	TAT	TTG	ACG	GAA	TTG	TAC	CGT	GAA	GCC	AAA	AAC	384
	Phe	Ser	Pro	Ala	Ala	Tyr	Leu	Thr	Glu	Leu	Tyr	Arg	Glu	Ala	Lys	Asn	
			115					120					125				
15	TTG	CAT	GAC	AGC	AGC	TCA	ATT	TAT	TAC	CTA	GAT	AAA	CGT	CGC	CCG	GAT	432
	Leu	His	Asp	Ser	Ser	Ser	Ile	Tyr	Tyr	Leu	Asp	Lys	Arg	Arg	Pro	Asp	
		130					135					140					
20	TTA	GCA	AGC	TTA	ATG	CTC	AGC	CAG	AAA	AAT	ATG	GAT	GAG	GAA	ATT	TCA	480
	Leu	Ala	Ser	Leu	Met	Leu	Ser	Gln	Lys	Asn	Met	Asp	Glu	Glu	Ile	Ser	
	145					150					155					160	
25	ACG	CTG	GCT	CTC	TCT	AAT	GAA	TTG	TGC	CTT	GCC	GGG	ATC	GAA	ACA	AAA	528
	Thr	Leu	Ala	Leu	Ser	Asn	Glu	Leu	Cys	Leu	Ala	Gly	Ile	Glu	Thr	Lys	
					165				170						175		
	ACA	GGA	AAA	TCA	CAA	GAT	GAA	GTG	ATG	GAT	ATG	TTG	TCA	ACT	TAT	CGT	576
30	Thr	Gly	Lys	Ser	Gln	Asp	Glu	Val	Met	Asp	Met	Leu	Ser	Thr	Tyr	Arg	
				180					185					190			
	TTA	AGT	GGA	GAG	ACA	CCT	TAT	CAT	CAC	GCT	TAT	GAA	ACT	GTT	CGT	GAA	624
	Leu	Ser	Gly	Glu	Thr	Pro	Tyr	His	His	Ala	Tyr	Glu	Thr	Val	Arg	Glu	
			195					200					205				
35	ATC	GTT	CAT	GAA	CGT	GAT	CCA	GGA	TTT	CGT	CAT	TTG	TCA	CAG	GCA	CCC	572
	Ile	Val	His	Glu	Arg	Asp	Pro	Gly	Phe	Arg	His	Leu	Ser	Gln	Ala	Pro	
		210					215					220					
40	ATT	GTT	GCT	GCT	AAG	CTC	GAT	CCT	GTG	ACT	TTG	TTG	GGT	ATT	AGC	TCC	720
	Ile	Val	Ala	Ala	Lys	Leu	Asp	Pro	Val	Thr	Leu	Leu	Gly	Ile	Ser	Ser	
	225					230					235					240	
45	CAT	ATT	TCG	CCA	GAA	CTG	TAT	AAC	TTG	CTG	ATT	GAG	GAG	ATC	CCG	GAA	768
	His	Ile	Ser	Pro	Glu	Leu	Tyr	Asn	Leu	Leu	Ile	Glu	Glu	Ile	Pro	Glu	
					245				250						255		
50	AAA	GAT	GAA	GCC	GCG	CTT	GAT	ACG	CTT	TAT	AAA	ACA	AAC	TTT	GGC	GAT	816
	Lys	Asp	Glu	Ala	Ala	Leu	Asp	Thr	Leu	Tyr	Lys	Thr	Asn	Phe	Gly	Asp	
				260					265					270			
	ATT	ACT	ACT	GCT	CAG	TTA	ATG	TCC	CCA	AGT	TAT	CTG	GCC	CGG	TAT	TAT	864
	Ile	Thr	Thr	Ala	Gln	Leu	Met	Ser	Pro	Ser	Tyr	Leu	Ala	Arg	Tyr	Tyr	
				275				280					285				
55	GGC	GTC	TCA	CCG	GAA	GAT	ATT	GCC	TAC	GTG	ACG	ACT	TCA	TTA	TCA	CAT	912
	Gly	Val	Ser	Pro	Glu	Asp	Ile	Ala	Tyr	Val	Thr	Thr	Ser	Leu	Ser	His	
		290					295					300					
60	GTT	GGA	TAT	AGC	AGT	GAT	ATT	CTG	GTT	ATT	CCG	TTG	GTC	GAT	GGT	GTG	960
	Val	Gly	Tyr	Ser	Ser	Asp	Ile	Leu	Val	Ile	Pro	Leu	Val	Asp	Gly	Val	
	305					310					315					320	
65	GGT	AAG	ATG	GAA	GTA	GTT	CGT	GTT	ACC	CGA	ACA	CCA	TCG	GAT	AAT	TAT	1008
	Gly	Lys	Met	Glu	Val	Val	Arg	Val	Thr	Arg	Thr	Pro	Ser	Asp	Asn	Tyr	
					325					330					335		

	ACC	AGT	CAG	ACG	AAT	TAT	ATT	GAG	CTG	TAT	CCA	CAG	GGT	GGC	GAC	AAT	1056
	Thr	Ser	Gln	Thr	Asn	Tyr	Ile	Glu	Leu	Tyr	Pro	Gln	Gly	Gly	Asp	Asn	
				340					345					350			
5	TAT	TTG	ATC	AAA	TAC	AAT	CTA	AGC	AAT	AGT	TTT	GGT	TTG	GAT	GAT	TTT	1104
	Tyr	Leu	Ile	Lys	Tyr	Asn	Leu	Ser	Asn	Ser	Phe	Gly	Leu	Asp	Asp	Phe	
			355					360					365				
10	TAT	CTG	CAA	TAT	AAA	GAT	GGT	TCC	GCT	GAT	TGG	ACT	GAG	ATT	GCC	CAT	1152
	Tyr	Leu	Gln	Tyr	Lys	Asp	Gly	Ser	Ala	Asp	Trp	Thr	Glu	Ile	Ala	His	
			370				375						380				
15	AAT	CCC	TAT	CCT	GAT	ATG	GTC	ATA	AAT	CAA	AAG	TAT	GAA	TCA	CAG	GCG	1200
	Asn	Pro	Tyr	Pro	Asp	Met	Val	Ile	Asn	Gln	Lys	Tyr	Glu	Ser	Gln	Ala	
	385					390					395					400	
20	ACA	ATC	AAA	CGT	AGT	GAC	TCT	GAC	AAT	ATA	CTC	AGT	ATA	GGG	TTA	CAA	1248
	Thr	Ile	Lys	Arg	Ser	Asp	Ser	Asp	Asn	Ile	Leu	Ser	Ile	Gly	Leu	Gln	
				405						410					415		
25	AGA	TGG	CAT	AGC	GGT	AGT	TAT	AAT	TTT	GCC	GCC	GCC	AAT	TTT	AAA	ATT	1296
	Arg	Trp	His	Ser	Gly	Ser	Tyr	Asn	Phe	Ala	Ala	Ala	Asn	Phe	Lys	Ile	
				420					425					430			
30	GAC	CAA	TAC	TCC	CCG	AAA	GCT	TTC	CTG	CTT	AAA	ATG	AAT	AAG	GCT	ATT	1344
	Asp	Gln	Tyr	Ser	Pro	Lys	Ala	Phe	Leu	Leu	Lys	Met	Asn	Lys	Ala	Ile	
			435				440					445					
35	CGG	TTG	CTC	AAA	GCT	ACC	GGC	CTC	TCT	TTT	GCT	ACG	TTG	GAG	CGT	ATT	1392
	Arg	Leu	Leu	Lys	Ala	Thr	Gly	Leu	Ser	Phe	Ala	Thr	Leu	Glu	Arg	Ile	
		450					455					460					
40	GTT	GAT	AGT	GTT	AAT	AGC	ACC	AAA	TCC	ATC	ACG	GTT	GAG	GTA	TTA	AAC	1440
	Val	Asp	Ser	Val	Asn	Ser	Thr	Lys	Ser	Ile	Thr	Val	Glu	Val	Leu	Asn	
	465				470						475					480	
45	AAG	GTT	TAT	CGG	GTA	AAA	TTC	TAT	ATT	GAT	CGT	TAT	GGC	ATC	AGT	GAA	1488
	Lys	Val	Tyr	Arg	Val	Lys	Phe	Tyr	Ile	Asp	Arg	Tyr	Gly	Ile	Ser	Glu	
				485						490					495		
50	GAG	ACA	GCC	GCT	ATT	TTG	GCT	AAT	ATT	AAT	ATC	TCT	CAG	CAA	GCT	GTT	1536
	Glu	Thr	Ala	Ala	Ile	Leu	Ala	Asn	Ile	Asn	Ile	Ser	Gln	Gln	Ala	Val	
				500					505					510			
55	GGC	AAT	CAG	CTT	AGC	CAG	TTT	GAG	CAA	CTA	TTT	AAT	CAC	CCG	CCG	CTC	1584
	Gly	Asn	Gln	Leu	Ser	Gln	Phe	Glu	Gln	Leu	Phe	Asn	His	Pro	Pro	Leu	
			515					520					525				
60	AAT	GGT	ATT	CGC	TAT	GAA	ATC	AGT	GAG	GAC	AAC	TCC	AAA	CAT	CTT	CCT	1632
	Asn	Gly	Ile	Arg	Tyr	Glu	Ile	Ser	Glu	Asp	Asn	Ser	Lys	His	Leu	Pro	
		530					535					540					
65	AAT	CCT	GAT	CTG	AAC	CTT	AAA	CCA	GAC	AGT	ACC	GGT	GAT	GAT	CAA	CGC	1680
	Asn	Pro	Asp	Leu	Asn	Leu	Lys	Pro	Asp	Ser	Thr	Gly	Asp	Asp	Gln	Arg	
	545					550					555					560	
70	AAG	GCG	GTT	TTA	AAA	CGC	GCG	TTT	CAG	GTT	AAC	GCC	AGT	GAG	TTG	TAT	1728
	Lys	Ala	Val	Leu	Lys	Arg	Ala	Phe	Gln	Val	Asn	Ala	Ser	Glu	Leu	Tyr	
				565					570						575		
75	CAG	ATG	TTA	TTG	ATC	ACT	GAT	CGT	AAA	GAA	GAC	GGT	GTT	ATC	AAA	AAT	1776
	Gln	Met	Leu	Leu	Ile	Thr	Asp	Arg	Lys	Glu	Asp	Gly	Val	Ile	Lys	Asn	
				580					585					590			
80	AAC	TTA	GAG	AAT	TTG	TCT	GAT	CTG	TAT	TTG	GTT	AGT	TTG	CTG	GCC	CAG	1824
	Asn	Leu	Glu	Asn	Leu	Ser	Asp	Leu	Tyr	Leu	Val	Ser	Leu	Leu	Ala	Gln	



	595					600					605						
5	ATT	CAT	AAC	CTG	ACT	ATT	GCT	GAA	TTG	AAC	ATT	TTG	TTG	GTG	ATT	TGT	1872
	Ile	His	Asn	Leu	Thr	Ile	Ala	Glu	Leu	Asn	Ile	Leu	Leu	Val	Ile	Cys	
		610					615					620					
10	GGC	TAT	GGC	GAC	ACC	AAC	ATT	TAT	CAG	ATT	ACC	GAC	GAT	AAT	TTA	GCC	1920
	Gly	Tyr	Gly	Asp	Thr	Asn	Ile	Tyr	Gln	Ile	Thr	Asp	Asp	Asn	Leu	Ala	
	625					630					635					640	
	AAA	ATA	GTG	GAA	ACA	TTG	TTG	TGG	ATC	ACT	CAA	TGG	TTG	AAG	ACC	CAA	1958
	Lys	Ile	Val	Glu	Thr	Leu	Leu	Trp	Ile	Thr	Gln	Trp	Leu	Lys	Thr	Gln	
					645					650					655		
15	AAA	TGG	ACA	GTT	ACC	GAC	CTG	TTT	CTG	ATG	ACC	ACG	GCC	ACT	TAC	AGC	2016
	Lys	Trp	Thr	Val	Thr	Asp	Leu	Phe	Leu	Met	Thr	Thr	Ala	Thr	Tyr	Ser	
					660				665					670			
20	ACC	ACT	TTA	ACG	CCA	GAA	ATT	AGC	AAT	CTG	ACG	GCT	ACG	TTG	TCT	TCA	2064
	Thr	Thr	Leu	Thr	Pro	Glu	Ile	Ser	Asn	Leu	Thr	Ala	Thr	Leu	Ser	Ser	
			675					680					685				
25	ACT	TTG	CAT	GGC	AAA	GAG	AGT	CTG	ATT	GGG	GAA	GAT	CTG	AAA	AGA	GCA	2112
	Thr	Leu	His	Gly	Lys	Glu	Ser	Leu	Ile	Gly	Glu	Asp	Leu	Lys	Arg	Ala	
		690					695					700					
30	ATG	GCG	CCT	TGC	TTC	ACT	TCG	GCT	TTG	CAT	TTG	ACT	TCT	CAA	GAA	GTT	2160
	Met	Ala	Pro	Cys	Phe	Thr	Ser	Ala	Leu	His	Leu	Thr	Ser	Gln	Glu	Val	
	705					710					715					720	
	GCG	TAT	GAC	CTG	CTG	TTG	TGG	ATA	GAC	CAG	ATT	CAA	CCG	GCA	CAA	ATA	2208
	Ala	Tyr	Asp	Leu	Leu	Leu	Trp	Ile	Asp	Gln	Ile	Gln	Pro	Ala	Gln	Ile	
					725					730					735		
35	ACT	GTT	GAT	GGG	TTT	TGG	GAA	GAA	GTG	CAA	ACA	ACA	CCA	ACC	AGC	TTG	2256
	Thr	Val	Asp	Gly	Phe	Trp	Glu	Glu	Val	Gln	Thr	Thr	Pro	Thr	Ser	Leu	
				740					745					750			
40	AAG	GTG	ATT	ACC	TTT	GCT	CAG	GTG	CTG	GCA	CAA	TTG	AGC	CTG	ATC	TAT	2304
	Lys	Val	Ile	Thr	Phe	Ala	Gln	Val	Leu	Ala	Gln	Leu	Ser	Leu	Ile	Tyr	
			755					760					765				
45	CGT	CGT	ATT	GGG	TTA	AGT	GAA	ACG	GAA	CTG	TCA	CTG	ATC	GTG	ACT	CAA	2352
	Arg	Arg	Ile	Gly	Leu	Ser	Glu	Thr	Glu	Leu	Ser	Leu	Ile	Val	Thr	Gln	
		770					775					780					
50	TCT	TCT	CTG	CTA	GTG	GCA	GGC	AAA	AGC	ATA	CTG	GAT	CAC	GGT	CTG	TTA	2400
	Ser	Ser	Leu	Leu	Val	Ala	Gly	Lys	Ser	Ile	Leu	Asp	His	Gly	Leu	Leu	
	785					790					795					800	
	ACC	CTG	ATG	GCC	TTG	GAA	GGT	TTT	CAT	ACC	TGG	GTT	AAT	GGC	TTG	GGG	2448
	Thr	Leu	Met	Ala	Leu	Glu	Gly	Phe	His	Thr	Trp	Val	Asn	Gly	Leu	Gly	
					805					810					815		
55	CAA	CAT	GCC	TCC	TTG	ATA	TTG	GCG	GCG	TTG	AAA	GAC	GGA	GCC	TTG	ACA	2496
	Gln	His	Ala	Ser	Leu	Ile	Leu	Ala	Ala	Leu	Lys	Asp	Gly	Ala	Leu	Thr	
				820					825					830			
60	GTT	ACC	GAT	GTA	GCA	CAA	GCT	ATG	AAT	AAG	GAG	GAA	TCT	CTC	CTA	CAA	2544
	Val	Thr	Asp	Val	Ala	Gln	Ala	Met	Asn	Lys	Glu	Glu	Ser	Leu	Leu	Gln	
			835					840					845				
65	ATG	GCA	GCT	AAT	CAG	GTG	GAG	AAG	GAT	CTA	ACA	AAA	CTG	ACC	AGT	TGG	2592
	Met	Ala	Ala	Asn	Gln	Val	Glu	Lys	Asp	Leu	Thr	Lys	Leu	Thr	Ser	Trp	
		850					855					860					

	ACA	CAG	ATT	GAC	GCT	ATT	CTG	CAA	TGG	TTA	CAG	ATG	TCT	TCG	GCC	TTG	2640
	Thr	Gln	Ile	Asp	Ala	Ile	Leu	Gln	Trp	Leu	Gln	Met	Ser	Ser	Ala	Leu	
	865					870					875					880	
5	GCG	GTT	TCT	CCA	CTG	GAT	CTG	GCA	GGG	ATG	ATG	GCC	CTG	AAA	TAT	GGG	2688
	Ala	Val	Ser	Pro	Leu	Asp	Leu	Ala	Gly	Met	Met	Ala	Leu	Lys	Tyr	Gly	
					885					890						895	
10	ATA	GAT	CAT	AAC	TAT	GCT	GCC	TGG	CAA	GCT	GCG	GCG	GCT	GCG	CTG	ATG	2736
	Ile	Asp	His	Asn	Tyr	Ala	Ala	Trp	Gln	Ala	Ala	Ala	Ala	Ala	Leu	Met	
				900					905						910		
15	GCT	GAT	CAT	GCT	AAT	CAG	GCA	CAG	AAA	AAA	CTG	GAT	GAG	ACG	TTC	AGT	2784
	Ala	Asp	His	Ala	Asn	Gln	Ala	Gln	Lys	Lys	Leu	Asp	Glu	Thr	Phe	Ser	
			915					920					925				
20	AAG	GCA	TTA	TGT	AAC	TAT	TAT	ATT	AAT	GCT	GTT	GTC	GAT	AGT	GCT	GCT	2832
	Lys	Ala	Leu	Cys	Asn	Tyr	Tyr	Ile	Asn	Ala	Val	Val	Asp	Ser	Ala	Ala	
		930					935					940					
25	GGA	GTA	CGT	GAT	CGT	AAC	GGT	TTA	TAT	ACC	TAT	TTG	CTG	ATT	GAT	AAT	2880
	Gly	Val	Arg	Asp	Arg	Asn	Gly	Leu	Tyr	Thr	Tyr	Leu	Leu	Ile	Asp	Asn	
	945					950					955					960	
30	CAG	GTT	TCT	GCC	GAT	GTG	ATC	ACT	TCA	CGT	ATT	GCA	GAA	GCT	ATC	GCC	2928
	Gln	Val	Ser	Ala	Asp	Val	Ile	Thr	Ser	Arg	Ile	Ala	Glu	Ala	Ile	Ala	
					965					970					975		
35	GGT	ATT	CAA	CTG	TAC	GTT	AAC	CGG	GCT	TTA	AAC	CGA	GAT	GAA	GGT	CAG	2976
	Gly	Ile	Gln	Leu	Tyr	Val	Asn	Arg	Ala	Leu	Asn	Arg	Asp	Glu	Gly	Gln	
				980					985					990			
40	CTT	GCA	TCG	GAC	GTT	AGT	ACC	CGT	CAG	TTC	TTC	ACT	GAC	TGG	GAA	CGT	3024
	Leu	Ala	Ser	Asp	Val	Ser	Thr	Arg	Gln	Phe	Phe	Thr	Asp	Trp	Glu	Arg	
			995					1000					1005				
45	TAC	AAT	AAA	CGT	TAC	AGT	ACT	TGG	GCT	GGT	GTC	TCT	GAA	CTG	GTC	TAT	3072
	Tyr	Asn	Lys	Arg	Tyr	Ser	Thr	Trp	Ala	Gly	Val	Ser	Glu	Leu	Val	Tyr	
		1010					1015					1020					
50	TAT	CCA	GAA	AAC	TAT	GTT	GAT	CCC	ACT	CAG	CGC	ATT	GGG	CAA	ACC	AAA	3120
	Tyr	Pro	Glu	Asn	Tyr	Val	Asp	Pro	Thr	Gln	Arg	Ile	Gly	Gln	Thr	Lys	
	1025					1030					1035					1040	
55	ATG	ATG	GAT	GCG	CTG	TTG	CAA	TCC	ATC	AAC	CAG	AGC	CAG	CTA	AAT	GCG	3158
	Met	Met	Asp	Ala	Leu	Leu	Gln	Ser	Ile	Asn	Gln	Ser	Gln	Leu	Asn	Ala	
					1045					1050					1055		
60	GAT	ACG	GTG	GAA	GAT	GCT	TTC	AAA	ACT	TAT	TTG	ACC	AGC	TTT	GAG	CAG	3216
	Asp	Thr	Val	Glu	Asp	Ala	Phe	Lys	Thr	Tyr	Leu	Thr	Ser	Phe	Glu	Gln	
				1060					1065					1070			
65	GTA	GCA	AAT	CTG	AAA	GTA	ATT	AGT	GCT	TAC	CAC	GAT	AAT	GTG	AAT	GTG	3264
	Val	Ala	Asn	Leu	Lys	Val	Ile	Ser	Ala	Tyr	His	Asp	Asn	Val	Asn	Val	
			1075					1080					1085				
70	GAT	CAA	GGA	TTA	ACT	TAT	TTT	ATC	GGT	ATC	GAC	CAA	GCA	GCT	CCG	GGT	3312
	Asp	Gln	Gly	Leu	Thr	Tyr	Phe	Ile	Gly	Ile	Asp	Gln	Ala	Ala	Pro	Gly	
		1090					1095					1100					
75	ACG	TAT	TAC	TGG	CGT	AGT	GTT	GAT	CAC	AGC	AAA	TGT	GAA	AAT	GGC	AAG	3350
	Thr	Tyr	Tyr	Trp	Arg	Ser	Val	Asp	His	Ser	Lys	Cys	Glu	Asn	Gly	Lys	
	1105					1110					1115					1120	
80	TTT	GCC	GCT	AAT	GCT	TGG	GGT	GAG	TGG	AAT	AAA	ATT	ACC	TGT	GCT	GTC	3408
	Phe	Ala	Ala	Asn	Ala	Trp	Gly	Glu	Trp	Asn	Lys	Ile	Thr	Cys	Ala	Val	

	1125	1130	1135	
5	AAT CCT TGG AAA AAT ATC ATC CGT CCG GTT GTT TAT ATG TCC CGC TTA 3456 Asn Pro Trp Lys Asn Ile Ile Arg Pro Val Val Tyr Met Ser Arg Leu 1140 1145 1150			
10	TAT CTG CTA TGG CTG GAG CAG CAA TCA AAG AAA AGT GAT GAT GGT AAA 3504 Tyr Leu Leu Trp Leu Glu Gln Gln Ser Lys Lys Ser Asp Asp Gly Lys 1155 1160 1165			
15	ACC ACG ATT TAT CAA TAT AAC TTA AAA CTG GCT CAT ATT CGT TAC GAC 3552 Thr Thr Ile Tyr Gln Tyr Asn Leu Lys Leu Ala His Ile Arg Tyr Asp 1170 1175 1180			
20	GGT AGT TGG AAT ACA CCA TTT ACT TTT GAT GTG ACA GAA AAG GTA AAA 3600 Gly Ser Trp Asn Thr Pro Phe Thr Phe Asp Val Thr Glu Lys Val Lys 1185 1190 1195 1200			
25	AAT TAC ACG TCG AGT ACT GAT GCT GCT GAA TCT TTA GGG TTG TAT TGT 3648 Asn Tyr Thr Ser Ser Thr Asp Ala Ala Glu Ser Leu Gly Leu Tyr Cys 1205 1210 1215			
30	ACT GGT TAT CAA GGG GAA GAC ACT CTA TTA GTT ATG TTC TAT TCG ATG 3696 Thr Gly Tyr Gln Gly Glu Asp Thr Leu Leu Val Met Phe Tyr Ser Met 1220 1225 1230			
35	CAG AGT AGT TAT AGC TCC TAT ACC GAT AAT AAT GCG CCG GTC ACT GGG 3744 Gln Ser Ser Tyr Ser Ser Tyr Thr Asp Asn Asn Ala Pro Val Thr Gly 1235 1240 1245			
40	CTA TAT ATT TTC GCT GAT ATG TCA TCA GAC AAT ATG ACG AAT GCA CAA 3792 Leu Tyr Ile Phe Ala Asp Met Ser Ser Asp Asn Met Thr Asn Ala Gln 1250 1255 1260			
45	GCA ACT AAC TAT TGG AAT AAC AGT TAT CCG CAA TTT GAT ACT GTG ATG 3840 Ala Thr Asn Tyr Trp Asn Asn Ser Tyr Pro Gln Phe Asp Thr Val Met 1265 1270 1275 1280			
50	GCA GAT CCG GAT AGC GAC AAT AAA AAA GTC ATA ACC AGA AGA GTT AAT 3888 Ala Asp Pro Asp Ser Asp Asn Lys Lys Val Ile Thr Arg Arg Val Asn 1285 1290 1295			
55	AAC CGT TAT GCG GAG GAT TAT GAA ATT CCT TCC TCT GTG ACA AGT AAC 3936 Asn Arg Tyr Ala Glu Asp Tyr Glu Ile Pro Ser Ser Val Thr Ser Asn 1300 1305 1310			
60	AGT AAT TAT TCT TGG GGT GAT CAC AGT TTA ACC ATG CTT TAT GGT GGT 3984 Ser Asn Tyr Ser Trp Gly Asp His Ser Leu Thr Met Leu Tyr Gly Gly 1315 1320 1325			
65	AGT GTT CCT AAT ATT ACT TTT GAA TCG GCG GCA GAA GAT TTA AGG CTA 4032 Ser Val Pro Asn Ile Thr Phe Glu Ser Ala Ala Glu Asp Leu Arg Leu 1330 1335 1340			
70	TCT ACC AAT ATG GCA TTG AGT ATT ATT CAT AAT GGA TAT GCG GGA ACC 4080 Ser Thr Asn Met Ala Leu Ser Ile Ile His Asn Gly Tyr Ala Gly Thr 1345 1350 1355 1360			
75	CGC CGT ATA CAA TGT AAT CTT ATG AAA CAA TAC GCT TCA TTA GGT GAT 4128 Arg Arg Ile Gln Cys Asn Leu Met Lys Gln Tyr Ala Ser Leu Gly Asp 1365 1370 1375			
80	AAA TTT ATA ATT TAT GAT TCA TCA TTT GAT GAT GCA AAC CGT TTT AAT 4176 Lys Phe Ile Ile Tyr Asp Ser Ser Phe Asp Asp Ala Asn Arg Phe Asn 1380 1385 1390			

	CTG	GTG	CCA	TTG	TTT	AAA	TTC	GGA	AAA	GAC	GAG	AAC	TCA	GAT	GAT	ACT	431
	Leu	Val	Pro	Leu	Phe	Lys	Phe	Gly	Lys	Asp	Glu	Asn	Ser	Asp	Asp	Ser	
			1395					1400					1405				
5	ATT	TGT	ATA	TAT	AAT	GAA	AAC	CCT	TCC	TCT	GAA	GAT	AAG	AAG	TGG	TAT	432
	Ile	Cys	Ile	Tyr	Asn	Glu	Asn	Pro	Ser	Ser	Glu	Asp	Lys	Lys	Trp	Tyr	
		1410					1415					1420					
10	TTT	TCT	TCG	AAA	GAT	GAC	AAT	AAA	ACA	GCG	GAT	TAT	AAT	GGT	GGA	ACT	433
	Phe	Ser	Ser	Lys	Asp	Asp	Asn	Lys	Thr	Ala	Asp	Tyr	Asn	Gly	Gly	Thr	
		1425				1430					1435					1440	
15	CAA	TGT	ATA	GAT	GCT	GGA	ACC	AGT	AAC	AAA	GAT	TTT	TAT	TAT	AAT	CTC	436
	Gln	Cys	Ile	Asp	Ala	Gly	Thr	Ser	Asn	Lys	Asp	Phe	Tyr	Tyr	Asn	Leu	
				1445						1450						1455	
20	CAG	GAG	ATT	GAA	GTA	ATT	AGT	GTT	ACT	GGT	GGG	TAT	TGG	TCG	AGT	TAT	441
	Gln	Glu	Ile	Glu	Val	Ile	Ser	Val	Thr	Gly	Gly	Tyr	Trp	Ser	Ser	Tyr	
				1460					1465					1470			
	AAA	ATA	TCC	AAC	CCG	ATT	AAT	ATC	AAT	ACG	GGC	ATT	GAT	AGT	GCT	AAA	446
	Lys	Ile	Ser	Asn	Pro	Ile	Asn	Ile	Asn	Thr	Gly	Ile	Asp	Ser	Ala	Lys	
			1475					1480					1485				
25	GTA	AAA	GTC	ACC	GTA	AAA	GCG	GGT	GGT	GAC	GAT	CAA	ATC	TTT	ACT	GCT	451
	Val	Lys	Val	Thr	Val	Lys	Ala	Gly	Gly	Asp	Asp	Gln	Ile	Phe	Thr	Ala	
		1490					1495					1500					
30	GAT	AAT	AGT	ACC	TAT	GTT	CCT	CAG	CAA	CCG	GCA	CCC	AGT	TTT	GAG	GAG	456
	Asp	Asn	Ser	Thr	Tyr	Val	Pro	Gln	Gln	Pro	Ala	Pro	Ser	Phe	Glu	Glu	
		1505				1510					1515					1520	
35	ATG	ATT	TAT	CAG	TTC	AAT	AAC	CTG	ACA	ATA	GAT	TGT	AAG	AAT	TTA	AAT	460
	Met	Ile	Tyr	Gln	Phe	Asn	Asn	Leu	Thr	Ile	Asp	Cys	Lys	Asn	Leu	Asn	
				1525					1530						1535		
40	TTC	ATC	GAC	AAT	CAG	GCA	CAT	ATT	GAG	ATT	GAT	TTC	ACC	GCT	ACG	GCA	465
	Phe	Ile	Asp	Asn	Gln	Ala	His	Ile	Glu	Ile	Asp	Phe	Thr	Ala	Thr	Ala	
				1540					1545					1550			
	CAA	GAT	GGC	CGA	TTC	TTG	GGT	GCA	GAA	ACT	TTT	ATT	ATC	CCG	GTA	ACT	470
	Gln	Asp	Gly	Arg	Phe	Leu	Gly	Ala	Glu	Thr	Phe	Ile	Ile	Pro	Val	Thr	
			1555					1560					1565				
45	AAA	AAA	GTT	CTC	GGT	ACT	GAG	AAC	GTG	ATT	GCG	TTA	TAT	AGC	GAA	AAT	475
	Lys	Lys	Val	Leu	Gly	Thr	Glu	Asn	Val	Ile	Ala	Leu	Tyr	Ser	Glu	Asn	
		1570					1575					1580					
50	AAC	GGT	GTT	CAA	TAT	ATG	CAA	ATT	GGC	GCA	TAT	CGT	ACC	CGT	TTG	AAT	480
	Asn	Gly	Val	Gln	Tyr	Met	Gln	Ile	Gly	Ala	Tyr	Arg	Thr	Arg	Leu	Asn	
		1585				1590					1595					1600	
55	ACG	TTA	TTC	GCT	CAA	CAG	TTG	GTT	AGC	CGT	GCT	AAT	CGT	GGC	ATT	GAT	484
	Thr	Leu	Phe	Ala	Gln	Gln	Leu	Val	Ser	Arg	Ala	Asn	Arg	Gly	Ile	Asp	
				1605						1610					1615		
60	GCA	GTG	CTC	AGT	ATG	GAA	ACT	CAG	AAT	ATT	CAG	GAA	CCG	CAA	TTA	GGA	489
	Ala	Val	Leu	Ser	Met	Glu	Thr	Gln	Asn	Ile	Gln	Glu	Pro	Gln	Leu	Gly	
				1620					1625					1630			
	GCG	GGC	ACA	TAT	GTG	CAG	CTT	GTG	TTG	GAT	AAA	TAT	GAT	GAG	TCT	ATT	494
	Ala	Gly	Thr	Tyr	Val	Gln	Leu	Val	Leu	Asp	Lys	Tyr	Asp	Glu	Ser	Ile	
			1635				1640						1645				
65	CAT	GGC	ACT	AAT	AAA	AGC	TTT	GCT	ATT	GAA	TAT	GTT	GAT	ATA	TTT	AAA	499
	His	Gly	Thr	Asn	Lys	Ser	Phe	Ala	Ile	Glu	Tyr	Val	Asp	Ile	Phe	Lys	

	1650	1655	1660	
5	GAG AAC GAT AGT TTT GTG ATT TAT CAA GGA GAA CTT AGC GAA ACA AGT 5040 Glu Asn Asp Ser Phe Val Ile Tyr Gln Gly Glu Leu Ser Glu Thr Ser 1665 1670 1675 1680			
10	CAA ACT GTT GTG AAA GTT TTC TTA TCC TAT TTT ATA GAG GCG ACT GGA 5088 Gln Thr Val Val Lys Val Phe Leu Ser Tyr Phe Ile Glu Ala Thr Gly 1685 1690 1695			
15	AAT AAG AAC CAC TTA TGG GTA CGT GCT AAA TAC CAA AAG GAA ACG ACT 5136 Asn Lys Asn His Leu Trp Val Arg Ala Lys Tyr Gln Lys Glu Thr Thr 1700 1705 1710			
20	GAT AAG ATC TTG TTC GAC CGT ACT GAT GAG AAA GAT CCG CAC GGT TGG 5184 Asp Lys Ile Leu Phe Asp Arg Thr Asp Glu Lys Asp Pro His Gly Trp 1715 1720 1725			
25	TTT CTC AGC GAC GAT CAC AAG ACC TTT AGT GGT CTC TCT TCC GCA CAG 5232 Phe Leu Ser Asp Asp His Lys Thr Phe Ser Gly Leu Ser Ser Ala Gln 1730 1735 1740			
30	GCA TTA AAG AAC GAC AGT GAA CCG ATG GAT TTC TCT GGC GCC AAT GGT 5280 Ala Leu Lys Asn Asp Ser Glu Pro Met Asp Phe Ser Gly Ala Asn Ala 1745 1750 1755 1760			
35	CTC TAT TTC TGG GAA CTG TTC TAT TAC ACG CCG ATG ATG ATG GCT CAT 5328 Leu Tyr Phe Trp Glu Leu Phe Tyr Tyr Thr Pro Met Met Met Ala His 1765 1770 1775			
40	CGT TTG TTG CAG GAA CAG AAT TTT GAT GCG GCG AAC CAT TGG TTC CGT 5376 Arg Leu Leu Gln Glu Gln Asn Phe Asp Ala Ala Asn His Trp Phe Arg 1780 1785 1790			
45	TAT GTC TGG AGT CCA TCC GGT TAT ATC GTT GAT GGT AAA ATT GCT ATC 5424 Tyr Val Trp Ser Pro Ser Gly Tyr Ile Val Asp Gly Lys Ile Ala Ile 1795 1800 1805			
50	TAC CAC TGG AAC GTG CGA CCG CTG GAA GAA GAC ACC AGT TGG AAT GCA 5472 Tyr His Trp Asn Val Arg Pro Leu Glu Glu Asp Thr Ser Trp Asn Ala 1810 1815 1820			
55	CAA CAA CTG GAC TCC ACC GAT CCA GAT GCT GTA GCC CAA GAT GAT CCG 5520 Gln Gln Leu Asp Ser Thr Asp Pro Asp Ala Val Ala Gln Asp Asp Pro 1825 1830 1835 1840			
60	ATG CAC TAC AAG GTG GCT ACC TTT ATG GCG ACG TTG GAT CTG CTA ATG 5568 Met His Tyr Lys Val Ala Thr Phe Met Ala Thr Leu Asp Leu Leu Met 1845 1850 1855			
65	GCC CGT GGT GAT GCT GCT TAC CGC CAG TTA GAG CGT GAT ACG TTG GCT 5616 Ala Arg Gly Asp Ala Ala Tyr Arg Gln Leu Glu Arg Asp Thr Leu Ala 1860 1865 1870			
70	GAA GCT AAA ATG TGG TAT ACA CAG GCG CTT AAT CTG TTG GGT GAT GAG 5664 Glu Ala Lys Met Trp Tyr Thr Gln Ala Leu Asn Leu Leu Gly Asp Glu 1875 1880 1885			
75	CCA CAA GTG ATG CTG AGT ACG ACT TGG GCT AAT CCA ACA TTG GGT AAT 5712 Pro Gln Val Met Leu Ser Thr Thr Trp Ala Asn Pro Thr Leu Gly Asn 1890 1895 1900			
80	GCT GCT TCA AAA ACC ACA CAG CAG GTT CGT CAG CAA GTG CTT ACC CAG 5760 Ala Ala Ser Lys Thr Thr Gln Gln Val Arg Gln Gln Val Leu Thr Gln 1905 1910 1915 1920			

	TTC	CGT	CTC	AAT	AGC	AGG	GTA	AAA	ACC	CCG	TTG	CTA	GGA	ACA	GCC	AAT	5303
	Leu	Arg	Leu	Asn	Ser	Arg	Val	Lys	Thr	Pro	Leu	Leu	Gly	Thr	Ala	Asn	
				1925						1930						1935	
5	TCG	CTG	ACC	GCT	TTA	TTG	CTG	CCG	CAG	GAA	AAT	AGC	AAG	CTC	AAA	GGC	5356
	Ser	Leu	Thr	Ala	Leu	Phe	Leu	Pro	Gln	Glu	Asn	Ser	Lys	Leu	Lys	Gly	
				1940					1945							1950	
10	TAC	TGG	CGG	ACA	CTG	GCG	CAG	CGT	ATG	TTT	AAT	TTA	CGT	CAT	AAT	CTG	5904
	Tyr	Trp	Arg	Thr	Leu	Ala	Gln	Arg	Met	Phe	Asn	Leu	Arg	His	Asn	Leu	
			1955					1960								1965	
15	TCG	ATT	GAC	GGC	CAG	CCG	CTC	TCC	TTG	CCG	CTG	TAT	GCT	AAA	CCG	GCT	5352
	Ser	Ile	Asp	Gly	Gln	Pro	Leu	Ser	Leu	Pro	Leu	Tyr	Ala	Lys	Pro	Ala	
			1970					1975								1980	
20	GAT	CCA	AAA	GCT	TTA	CTG	AGT	GCG	GCG	GTT	TCA	GCT	TCT	CAA	GGG	GGA	6000
	Asp	Pro	Lys	Ala	Leu	Leu	Ser	Ala	Ala	Val	Ser	Ala	Ser	Gln	Gly	Gly	
			1985				1990					1995				2000	
	GCC	GAC	TTG	CCG	AAG	GCG	CCG	CTG	ACT	ATT	CAC	CGC	TTC	CCT	CAA	ATG	6048
	Ala	Asp	Leu	Pro	Lys	Ala	Pro	Leu	Thr	Ile	His	Arg	Phe	Pro	Gln	Met	
					2005						2010					2015	
25	CTA	GAA	GGG	GCA	CGG	GGC	TTG	GTT	AAC	CAG	CTT	ATA	CAG	TTC	GGT	AGT	6096
	Leu	Glu	Gly	Ala	Arg	Gly	Leu	Val	Asn	Gln	Leu	Ile	Gln	Phe	Gly	Ser	
				2020					2025							2030	
30	TCA	CTA	TTG	GGG	TAC	AGT	GAG	CGT	CAG	GAT	GCG	GAA	GCT	ATG	AGT	CAA	6144
	Ser	Leu	Leu	Gly	Tyr	Ser	Glu	Arg	Gln	Asp	Ala	Glu	Ala	Met	Ser	Gln	
				2035				2040						2045			
35	CTA	CTG	CAA	ACC	CAA	GCC	AGC	GAG	TTA	ATA	CTG	ACC	AGT	ATT	CGT	ATG	6192
	Leu	Leu	Gln	Thr	Gln	Ala	Ser	Glu	Leu	Ile	Leu	Thr	Ser	Ile	Arg	Met	
			2050				2055						2060				
40	CAG	GAT	AAC	CAA	TTG	GCA	GAG	CTG	GAT	TCG	GAA	AAA	ACC	GCC	TTG	CAA	6240
	Gln	Asp	Asn	Gln	Leu	Ala	Glu	Leu	Asp	Ser	Glu	Lys	Thr	Ala	Leu	Gln	
			2065				2070					2075				2080	
	GTC	TCT	TTA	GCT	GGA	GTG	CAA	CAA	CGG	TTT	GAC	AGC	TAT	AGC	CAA	CTG	6288
	Val	Ser	Leu	Ala	Gly	Val	Gln	Gln	Arg	Phe	Asp	Ser	Tyr	Ser	Gln	Leu	
					2085						2090					2095	
45	TAT	GAG	GAG	AAC	ATC	AAC	GCA	GGT	GAG	CAG	CGA	GCG	CTG	GCG	TTA	CGC	6336
	Tyr	Glu	Glu	Asn	Ile	Asn	Ala	Gly	Glu	Gln	Arg	Ala	Leu	Ala	Leu	Arg	
				2100					2105							2110	
50	TCA	GAA	TCT	GCT	ATT	GAG	TCT	CAG	GGA	GCG	CAG	ATT	TCC	CGT	ATG	GCA	6384
	Ser	Glu	Ser	Ala	Ile	Glu	Ser	Gln	Gly	Ala	Gln	Ile	Ser	Arg	Met	Ala	
				2115				2120						2125			
55	GGC	GCG	GGT	GTT	GAT	ATG	GCA	CCA	AAT	ATC	TTC	GCG	CTG	GCT	GAT	GGC	6432
	Gly	Ala	Gly	Val	Asp	Met	Ala	Pro	Asn	Ile	Phe	Gly	Leu	Ala	Asp	Gly	
			2130					2135					2140				
60	GGC	ATG	CAT	TAT	GGT	GCT	ATT	GCC	TAT	GCC	ATC	GCT	GAC	GGT	ATT	GAG	6480
	Gly	Met	His	Tyr	Gly	Ala	Ile	Ala	Tyr	Ala	Ile	Ala	Asp	Gly	Ile	Glu	
						2145		2150				2155				2160	
	TTG	AGT	GCT	TCT	GCC	AAG	ATG	GTT	GAT	GCG	GAG	AAA	GTT	GCT	CAG	TCG	6528
	Leu	Ser	Ala	Ser	Ala	Lys	Met	Val	Asp	Ala	Glu	Lys	Val	Ala	Gln	Ser	
						2165				2170						2175	
65	GAA	ATA	TAT	CGC	CGT	CGC	CGT	CAA	GAA	TGG	AAA	ATT	CAG	CGT	GAC	AAC	6576
	Glu	Ile	Tyr	Arg	Arg	Arg	Arg	Gln	Glu	Trp	Lys	Ile	Gln	Arg	Asp	Asn	

	2180	2185	2190
5	GCA CAA GCG GAG ATT AAC CAG TTA AAC GCG CAA CTG GAA TCA CTG TCT 6624 Ala Gln Ala Glu Ile Asn Gln Leu Asn Ala Gln Leu Glu Ser Leu Ser 2195 2200 2205		
10	ATT GCG CGT GAA GCC GGT GAA ATG CAA AAA GAG TAC CTG AAA ACC CAG 6672 Ile Arg Arg Glu Ala Ala Glu Met Gln Lys Glu Tyr Leu Lys Thr Gln 2210 2215 2220		
15	CAA GCT CAG GCG CAG GCA CAA CTT ACT TTC TTA AGA AGC AAA TTC AGT 6720 Gln Ala Gln Ala Gln Ala Gln Leu Thr Phe Leu Arg Ser Lys Phe Ser 2225 2230 2235 2240		
20	AAT CAA GCG TTA TAT AGT TGG TTA CGA GGG CGT TTG TCA GGT ATT TAT 6768 Asn Gln Ala Leu Tyr Ser Trp Leu Arg Gly Arg Leu Ser Gly Ile Tyr 2245 2250 2255		
25	TTC CAG TTC TAT GAC TTG GCC GTA TCA CGT TGC CTG ATG GCA GAG CAA 6816 Phe Gln Phe Tyr Asp Leu Ala Val Ser Arg Cys Leu Met Ala Glu Gln 2260 2265 2270		
30	TCC TAT CAA TGG GAA GCT AAT GAT AAT TCC ATT AGC TTT GTC AAA CCG 6864 Ser Tyr Gln Trp Glu Ala Asn Asp Asn Ser Ile Ser Phe Val Lys Pro 2275 2280 2285		
35	GGT GCA TGG CAA GGA ACT TAC GCC GGC TTA TTG TGT GGA GAA GCT TTG 6912 Gly Ala Trp Gln Gly Thr Tyr Ala Gly Leu Leu Cys Gly Glu Ala Leu 2290 2295 2300		
40	ATA CAA AAT CTG GCA CAA ATG GAA GAG GCA TAT CTG AAA TGG GAA TCT 6960 Ile Gln Asn Leu Ala Gln Met Glu Glu Ala Tyr Leu Lys Trp Glu Ser 2305 2310 2315 2320		
45	CGC GCT TTG GAA GTA GAA CGC ACG GTT TCA TTG GCA GTG GTT TAT GAT 7008 Arg Ala Leu Glu Val Glu Arg Thr Val Ser Leu Ala Val Val Tyr Asp 2325 2330 2335		
50	TCA CTG GAA GGT AAT GAT CGT TTT AAT TTA GCG GAA CAA ATA CCT GCA 7056 Ser Leu Glu Gly Asn Asp Arg Phe Asn Leu Ala Glu Gln Ile Pro Ala 2340 2345 2350		
55	TTA TTG GAT AAG GGG GAG GGA ACA GCA GGA ACT AAA GAA AAT GGG TTA 7104 Leu Leu Asp Lys Gly Glu Gly Thr Ala Gly Thr Lys Glu Asn Gly Leu 2355 2360 2365		
60	TCA TTG GCT AAT GCT ATC CTG TCA GCT TCG GTC AAA TTG TCC GAC TTG 7152 Ser Leu Ala Asn Ala Ile Leu Ser Ala Ser Val Lys Leu Ser Asp Leu 2370 2375 2380		
65	AAA CTG GGA ACG GAT TAT CCA GAC AGT ATC GTT GGT AGC AAC AAG GTT 7200 Lys Leu Gly Thr Asp Tyr Pro Asp Ser Ile Val Gly Ser Asn Lys Val 2385 2390 2395 2400		
70	CGT CGT ATT AAG CAA ATC AGT GTT TCG CTA CCT GCA TTG GTT GGG CCT 7248 Arg Arg Ile Lys Gln Ile Ser Val Ser Leu Pro Ala Leu Val Gly Pro 2405 2410 2415		
75	TAT CAG GAT GTT CAG GCT ATG CTC AGC TAT GGT GGC AGT ACT CAA TTG 7296 Tyr Gln Asp Val Gln Ala Met Leu Ser Tyr Gly Gly Ser Thr Gln Leu 2420 2425 2430		
80	CCG AAA GGT TGT TCA GCG TTG GCT GTG TCT CAT GGT ACC AAT GAT AGT 7344 Pro Lys Gly Cys Ser Ala Leu Ala Val Ser His Gly Thr Asn Asp Ser 2435 2440 2445		

GGT CAG TTC CAG TTG GAT TTC AAT GAC GGC AAA TAC CTG CCA TTT GAA 7392  
 Gly Gln Phe Gln Leu Asp Phe Asn Asp Gly Lys Tyr Leu Pro Phe Glu  
 2450 2455 2460  
 5 GGT ATT GCT CTT GAT GAT CAG GGT ACA CTG AAT CTT CAA TTT CCG AAT 7440  
 Gly Ile Ala Leu Asp Asp Gln Gly Thr Leu Asn Leu Gln Phe Pro Asn  
 2465 2470 2475 2480  
 10 GCT ACC GAC AAG CAG AAA GCA ATA TTG CAA ACT ATG AGC GAT ATT ATT 7488  
 Ala Thr Asp Lys Gln Lys Ala Ile Leu Gln Thr Met Ser Asp Ile Ile  
 2485 2490 2495  
 TTG CAT ATT CGT TAT ACC ATC CGT TAA 7515  
 Leu His Ile Arg Tyr Thr Ile Arg \*  
 15 2500 2505

## (2) INFORMATION FOR SEQ ID NO:12:

20 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 2505 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

30 Met Gln Asn Ser Leu Ser Ser Thr Ile Asp Thr Ile Cys Gln Lys Leu  
 1 5 10 15  
 Gln Leu Thr Cys Pro Ala Glu Ile Ala Leu Tyr Pro Phe Asp Thr Phe  
 20 25 30  
 35 Arg Glu Lys Thr Arg Gly Met Val Asn Trp Gly Glu Ala Lys Arg Ile  
 35 40 45  
 Tyr Glu Ile Ala Gln Ala Glu Gln Asp Arg Asn Leu Leu His Glu Lys  
 40 50 55 60  
 Arg Ile Phe Ala Tyr Ala Asn Pro Leu Leu Lys Asn Ala Val Arg Leu  
 65 70 75 80  
 45 Gly Thr Arg Gln Met Leu Gly Phe Ile Gln Gly Tyr Ser Asp Leu Phe  
 85 90 95  
 Gly Asn Arg Ala Asp Asn Tyr Ala Ala Pro Gly Ser Val Ala Ser Met  
 100 105 110  
 50 Phe Ser Pro Ala Ala Tyr Leu Thr Glu Leu Tyr Arg Glu Ala Lys Asn  
 115 120 125  
 Leu His Asp Ser Ser Ser Ile Tyr Tyr Leu Asp Lys Arg Arg Pro Asp  
 130 135 140  
 Leu Ala Ser Leu Met Leu Ser Gln Lys Asn Met Asp Glu Glu Ile Ser  
 145 150 155 160  
 60 Thr Leu Ala Leu Ser Asn Glu Leu Cys Leu Ala Gly Ile Glu Thr Lys  
 165 170 175  
 Thr Gly Lys Ser Gln Asp Glu Val Met Asp Met Leu Ser Thr Tyr Arg  
 180 185 190  
 65



	Leu	Ser	Gly	Glu	Thr	Pro	Tyr	His	His	Ala	Tyr	Glu	Thr	Val	Arg	Glu	
			195					200					205				
5	Ile	Val	His	Glu	Arg	Asp	Pro	Gly	Phe	Arg	His	Leu	Ser	Gln	Ala	Pro	
		210					215					220					
	Ile	Val	Ala	Ala	Lys	Leu	Asp	Pro	Val	Thr	Leu	Leu	Gly	Ile	Ser	Ser	
		225				230					235					240	
10	His	Ile	Ser	Pro	Glu	Leu	Tyr	Asn	Leu	Leu	Ile	Glu	Glu	Ile	Pro	Glu	
					245					250					255		
	Lys	Asp	Glu	Ala	Ala	Leu	Asp	Thr	Leu	Tyr	Lys	Thr	Asn	Phe	Gly	Asp	
				260					265					270			
15	Ile	Thr	Thr	Ala	Gln	Leu	Met	Ser	Pro	Ser	Tyr	Leu	Ala	Arg	Tyr	Tyr	
			275					280					285				
	Gly	Val	Ser	Pro	Glu	Asp	Ile	Ala	Tyr	Val	Thr	Thr	Ser	Leu	Ser	His	
20		290					295					300					
	Val	Gly	Tyr	Ser	Ser	Asp	Ile	Leu	Val	Ile	Pro	Leu	Val	Asp	Gly	Val	
		305				310				315						320	
25	Gly	Lys	Met	Glu	Val	Val	Arg	Val	Thr	Arg	Thr	Pro	Ser	Asp	Asn	Tyr	
				325					330						335		
	Thr	Ser	Gln	Thr	Asn	Tyr	Ile	Glu	Leu	Tyr	Pro	Gln	Gly	Gly	Asp	Asn	
				340					345					350			
30	Tyr	Leu	Ile	Lys	Tyr	Asn	Leu	Ser	Asn	Ser	Phe	Gly	Leu	Asp	Asp	Phe	
			355					360					365				
	Tyr	Leu	Gln	Tyr	Lys	Asp	Gly	Ser	Ala	Asp	Trp	Thr	Glu	Ile	Ala	His	
35		370					375					380					
	Asn	Pro	Tyr	Pro	Asp	Met	Val	Ile	Asn	Gln	Lys	Tyr	Glu	Ser	Gln	Ala	
		385				390					395					400	
40	Thr	Ile	Lys	Arg	Ser	Asp	Ser	Asp	Asn	Ile	Leu	Ser	Ile	Gly	Leu	Gln	
				405					410						415		
	Arg	Trp	His	Ser	Gly	Ser	Tyr	Asn	Phe	Ala	Ala	Ala	Asn	Phe	Lys	Ile	
				420					425					430			
45	Asp	Gln	Tyr	Ser	Pro	Lys	Ala	Phe	Leu	Leu	Lys	Met	Asn	Lys	Ala	Ile	
			435					440					445				
	Arg	Leu	Leu	Lys	Ala	Thr	Gly	Leu	Ser	Phe	Ala	Thr	Leu	Glu	Arg	Ile	
50		450					455					460					
	Val	Asp	Ser	Val	Asn	Ser	Thr	Lys	Ser	Ile	Thr	Val	Glu	Val	Leu	Asn	
		465				470					475					480	
55	Lys	Val	Tyr	Arg	Val	Lys	Phe	Tyr	Ile	Asp	Arg	Tyr	Gly	Ile	Ser	Glu	
				485					490					495			
	Glu	Thr	Ala	Ala	Ile	Leu	Ala	Asn	Ile	Asn	Ile	Ser	Gln	Gln	Ala	Val	
				500				505					510				
60	Gly	Asn	Gln	Leu	Ser	Gln	Phe	Glu	Gln	Leu	Phe	Asn	His	Pro	Pro	Leu	
			515					520					525				
	Asn	Gly	Ile	Arg	Tyr	Glu	Ile	Ser	Glu	Asp	Asn	Ser	Lys	His	Leu	Pro	
65		530					535				540						

	Asn	Pro	Asp	Leu	Asn	Leu	Lys	Pro	Asp	Ser	Thr	Gly	Asp	Asp	Gln	Arg	
	545					550					555					560	
5	Lys	Ala	Val	Leu	Lys	Arg	Ala	Phe	Gln	Val	Asn	Ala	Ser	Glu	Leu	Tyr	
					565					570					575		
	Gln	Met	Leu	Leu	Ile	Thr	Asp	Arg	Lys	Glu	Asp	Gly	Val	Ile	Lys	Asn	
				580					585					590			
10	Asn	Leu	Glu	Asn	Leu	Ser	Asp	Leu	Tyr	Leu	Val	Ser	Leu	Leu	Ala	Gln	
			595					600					605				
	Ile	His	Asn	Leu	Thr	Ile	Ala	Glu	Leu	Asn	Ile	Leu	Leu	Val	Ile	Cys	
	610						615					620					
15	Gly	Tyr	Gly	Asp	Thr	Asn	Ile	Tyr	Gln	Ile	Thr	Asp	Asp	Asn	Leu	Ala	
	625					630					635					640	
	Lys	Ile	Val	Glu	Thr	Leu	Leu	Trp	Ile	Thr	Gln	Trp	Leu	Lys	Thr	Gln	
20					645					650							
	Lys	Trp	Thr	Val	Thr	Asp	Leu	Phe	Leu	Met	Thr	Thr	Ala	Thr	Tyr	Ser	
				660					665					670			
25	Thr	Thr	Leu	Thr	Pro	Glu	Ile	Ser	Asn	Leu	Thr	Ala	Thr	Leu	Ser	Ser	
			675					680					685				
	Thr	Leu	His	Gly	Lys	Glu	Ser	Leu	Ile	Gly	Glu	Asp	Leu	Lys	Arg	Ala	
	690						695					700					
30	Met	Ala	Pro	Cys	Phe	Thr	Ser	Ala	Leu	His	Leu	Thr	Ser	Gln	Glu	Val	
	705					710					715					720	
	Ala	Tyr	Asp	Leu	Leu	Leu	Trp	Ile	Asp	Gln	Ile	Gln	Pro	Ala	Gln	Ile	
35					725					730					735		
	Thr	Val	Asp	Gly	Phe	Trp	Glu	Glu	Val	Gln	Thr	Thr	Pro	Thr	Ser	Leu	
				740					745					750			
40	Lys	Val	Ile	Thr	Phe	Ala	Gln	Val	Leu	Ala	Gln	Leu	Ser	Leu	Ile	Tyr	
		755					760						765				
	Arg	Arg	Ile	Gly	Leu	Ser	Glu	Thr	Glu	Leu	Ser	Leu	Ile	Val	Thr	Gln	
	770						775					780					
45	Ser	Ser	Leu	Leu	Val	Ala	Gly	Lys	Ser	Ile	Leu	Asp	His	Gly	Leu	Leu	
	785					790					795					800	
	Thr	Leu	Met	Ala	Leu	Glu	Gly	Phe	His	Thr	Trp	Val	Asn	Gly	Leu	Gly	
50					805					810					815		
	Gln	His	Ala	Ser	Leu	Ile	Leu	Ala	Ala	Leu	Lys	Asp	Gly	Ala	Leu	Thr	
				820					825					830			
55	Val	Thr	Asp	Val	Ala	Gln	Ala	Met	Asn	Lys	Glu	Glu	Ser	Leu	Leu	Gln	
			835					840					845				
	Met	Ala	Ala	Asn	Gln	Val	Glu	Lys	Asp	Leu	Thr	Lys	Leu	Thr	Ser	Trp	
	850						855					860					
60	Thr	Gln	Ile	Asp	Ala	Ile	Leu	Gln	Trp	Leu	Gln	Met	Ser	Ser	Ala	Leu	
	865					870					875					880	
	Ala	Val	Ser	Pro	Leu	Asp	Leu	Ala	Gly	Met	Met	Ala	Leu	Lys	Tyr	Gly	
65					885					890					895		

Ile Asp His Asn Tyr Ala Ala Trp Gln Ala Ala Ala Ala Ala Leu Met  
 900 905 910  
 5 Ala Asp His Ala Asn Gln Ala Gln Lys Lys Leu Asp Glu Thr Phe Ser  
 915 920 925  
 Lys Ala Leu Cys Asn Tyr Tyr Ile Asn Ala Val Val Asp Ser Ala Ala  
 930 935 940  
 10 Gly Val Arg Asp Arg Asn Gly Leu Tyr Thr Tyr Leu Leu Ile Asp Asn  
 945 950 955 960  
 Gln Val Ser Ala Asp Val Ile Thr Ser Arg Ile Ala Glu Ala Ile Ala  
 965 970 975  
 15 Gly Ile Gln Leu Tyr Val Asn Arg Ala Leu Asn Arg Asp Glu Gly Gln  
 980 985 990  
 Leu Ala Ser Asp Val Ser Thr Arg Gln Phe Phe Thr Asp Trp Glu Arg  
 995 1000 1005  
 20 Tyr Asn Lys Arg Tyr Ser Thr Trp Ala Gly Val Ser Glu Leu Val Tyr  
 1010 1015 1020  
 25 Tyr Pro Glu Asn Tyr Val Asp Pro Thr Gln Arg Ile Gly Gln Thr Lys  
 1025 1030 1035 1040  
 Met Met Asp Ala Leu Leu Gln Ser Ile Asn Gln Ser Gln Leu Asn Ala  
 1045 1050 1055  
 30 Asp Thr Val Glu Asp Ala Phe Lys Thr Tyr Leu Thr Ser Phe Glu Gln  
 1060 1065 1070  
 Val Ala Asn Leu Lys Val Ile Ser Ala Tyr His Asp Asn Val Asn Val  
 1075 1080 1085  
 35 Asp Gln Gly Leu Thr Tyr Phe Ile Gly Ile Asp Gln Ala Ala Pro Gly  
 1090 1095 1100  
 40 Thr Tyr Tyr Trp Arg Ser Val Asp His Ser Lys Cys Glu Asn Gly Lys  
 1105 1110 1115 1120  
 Phe Ala Ala Asn Ala Trp Gly Glu Trp Asn Lys Ile Thr Cys Ala Val  
 1125 1130 1135  
 45 Asn Pro Trp Lys Asn Ile Ile Arg Pro Val Val Tyr Met Ser Arg Leu  
 1140 1145 1150  
 Tyr Leu Leu Trp Leu Glu Gln Gln Ser Lys Lys Ser Asp Asp Gly Lys  
 1155 1160 1165  
 50 Thr Thr Ile Tyr Gln Tyr Asn Leu Lys Leu Ala His Ile Arg Tyr Asp  
 1170 1175 1180  
 55 Gly Ser Trp Asn Thr Pro Phe Thr Phe Asp Val Thr Glu Lys Val Lys  
 1185 1190 1195 1200  
 Asn Tyr Thr Ser Ser Thr Asp Ala Ala Glu Ser Leu Gly Leu Tyr Cys  
 1205 1210 1215  
 60 Thr Gly Tyr Gln Gly Glu Asp Thr Leu Leu Val Met Phe Tyr Ser Met  
 1220 1225 1230  
 Gln Ser Ser Tyr Ser Ser Tyr Thr Asp Asn Asn Ala Pro Val Thr Gly  
 1235 1240 1245  
 65

Leu Tyr Ile Phe Ala Asp Met Ser Ser Asp Asn Met Thr Asn Ala Gln  
 1250 1255 1260  
 5 Ala Thr Asn Tyr Trp Asn Asn Ser Tyr Pro Gln Phe Asp Thr Val Met  
 1265 1270 1275 1280  
 Ala Asp Pro Asp Ser Asp Asn Lys Lys Val Ile Thr Arg Arg Val Asn  
 1285 1290 1295  
 10 Asn Arg Tyr Ala Glu Asp Tyr Glu Ile Pro Ser Ser Val Thr Ser Asn  
 1300 1305 1310  
 Ser Asn Tyr Ser Trp Gly Asp His Ser Leu Thr Met Leu Tyr Gly Gly  
 1315 1320 1325  
 15 Ser Val Pro Asn Ile Thr Phe Glu Ser Ala Ala Glu Asp Leu Arg Leu  
 1330 1335 1340  
 Ser Thr Asn Met Ala Leu Ser Ile Ile His Asn Gly Tyr Ala Gly Thr  
 1345 1350 1355 1360  
 Arg Arg Ile Gln Cys Asn Leu Met Lys Gln Tyr Ala Ser Leu Gly Asp  
 1365 1370 1375  
 25 Lys Phe Ile Ile Tyr Asp Ser Ser Phe Asp Asp Ala Asn Arg Phe Asn  
 1380 1385 1390  
 Leu Val Pro Leu Phe Lys Phe Gly Lys Asp Glu Asn Ser Asp Asp Ser  
 1395 1400 1405  
 30 Ile Cys Ile Tyr Asn Glu Asn Pro Ser Ser Glu Asp Lys Lys Trp Tyr  
 1410 1415 1420  
 Phe Ser Ser Lys Asp Asp Asn Lys Thr Ala Asp Tyr Asn Gly Gly Thr  
 1425 1430 1435 1440  
 Gln Cys Ile Asp Ala Gly Thr Ser Asn Lys Asp Phe Tyr Tyr Asn Leu  
 1445 1450 1455  
 40 Gln Glu Ile Glu Val Ile Ser Val Thr Gly Gly Tyr Trp Ser Ser Tyr  
 1460 1465 1470  
 Lys Ile Ser Asn Pro Ile Asn Ile Asn Thr Gly Ile Asp Ser Ala Lys  
 1475 1480 1485  
 45 Val Lys Val Thr Val Lys Ala Gly Gly Asp Asp Gln Ile Phe Thr Ala  
 1490 1495 1500  
 Asp Asn Ser Thr Tyr Val Pro Gln Gln Pro Ala Pro Ser Phe Glu Glu  
 1505 1510 1515 1520  
 Met Ile Tyr Gln Phe Asn Asn Leu Thr Ile Asp Cys Lys Asn Leu Asn  
 1525 1530 1535  
 55 Phe Ile Asp Asn Gln Ala His Ile Glu Ile Asp Phe Thr Ala Thr Ala  
 1540 1545 1550  
 Gln Asp Gly Arg Phe Leu Gly Ala Glu Thr Phe Ile Ile Pro Val Thr  
 1555 1560 1565  
 60 Lys Lys Val Leu Gly Thr Glu Asn Val Ile Ala Leu Tyr Ser Glu Asn  
 1570 1575 1580  
 Asn Gly Val Gln Tyr Met Gln Ile Gly Ala Tyr Arg Thr Arg Leu Asn  
 1585 1590 1595 1600

Thr Leu Phe Ala Gln Gln Leu Val Ser Arg Ala Asn Arg Gly Ile Asp  
 1605 1610 1615  
 5 Ala Val Leu Ser Met Glu Thr Gln Asn Ile Gln Glu Pro Gln Leu Gly  
 1620 1625 1630  
 Ala Gly Thr Tyr Val Gln Leu Val Leu Asp Lys Tyr Asp Glu Ser Ile  
 1635 1640 1645  
 10 His Gly Thr Asn Lys Ser Phe Ala Ile Glu Tyr Val Asp Ile Phe Lys  
 1650 1655 1660  
 Glu Asn Asp Ser Phe Val Ile Tyr Gln Gly Glu Leu Ser Glu Thr Ser  
 1665 1670 1675 1680  
 15 Gln Thr Val Val Lys Val Phe Leu Ser Tyr Phe Ile Glu Ala Thr Gly  
 1685 1690 1695  
 Asn Lys Asn His Leu Trp Val Arg Ala Lys Tyr Gln Lys Glu Thr Thr  
 1700 1705 1710  
 Asp Lys Ile Leu Phe Asp Arg Thr Asp Glu Lys Asp Pro His Gly Trp  
 1715 1720 1725  
 25 Phe Leu Ser Asp Asp His Lys Thr Phe Ser Gly Leu Ser Ser Ala Gln  
 1730 1735 1740  
 Ala Leu Lys Asn Asp Ser Glu Pro Met Asp Phe Ser Gly Ala Asn Ala  
 1745 1750 1755 1760  
 30 Leu Tyr Phe Trp Glu Leu Phe Tyr Tyr Thr Pro Met Met Met Ala His  
 1765 1770 1775  
 Arg Leu Leu Gln Glu Gln Asn Phe Asp Ala Ala Asn His Trp Phe Arg  
 1780 1785 1790  
 35 Tyr Val Trp Ser Pro Ser Gly Tyr Ile Val Asp Gly Lys Ile Ala Ile  
 1795 1800 1805  
 40 Tyr His Trp Asn Val Arg Pro Leu Glu Glu Asp Thr Ser Trp Asn Ala  
 1810 1815 1820  
 Gln Gln Leu Asp Ser Thr Asp Pro Asp Ala Val Ala Gln Asp Asp Pro  
 1825 1830 1835 1840  
 45 Met His Tyr Lys Val Ala Thr Phe Met Ala Thr Leu Asp Leu Leu Met  
 1845 1850 1855  
 Ala Arg Gly Asp Ala Ala Tyr Arg Gln Leu Glu Arg Asp Thr Leu Ala  
 1860 1865 1870  
 50 Glu Ala Lys Met Trp Tyr Thr Gln Ala Leu Asn Leu Leu Gly Asp Glu  
 1875 1880 1885  
 Pro Gln Val Met Leu Ser Thr Thr Trp Ala Asn Pro Thr Leu Gly Asn  
 1890 1895 1900  
 Ala Ala Ser Lys Thr Thr Gln Gln Val Arg Gln Gln Val Leu Thr Gln  
 1905 1910 1915 1920  
 60 Leu Arg Leu Asn Ser Arg Val Lys Thr Pro Leu Leu Gly Thr Ala Asn  
 1925 1930 1935  
 Ser Leu Thr Ala Leu Phe Leu Pro Gln Glu Asn Ser Lys Leu Lys Gly  
 1940 1945 1950  
 65

Tyr Trp Arg Thr Leu Ala Gln Arg Met Phe Asn Leu Arg His Asn Leu  
 1955 1960 1965  
 5 Ser Ile Asp Gly Gln Pro Leu Ser Leu Pro Leu Tyr Ala Lys Pro Ala  
 1970 1975 1980  
 Asp Pro Lys Ala Leu Leu Ser Ala Ala Val Ser Ala Ser Gln Gly Gly  
 1985 1990 1995 2000  
 10 Ala Asp Leu Pro Lys Ala Pro Leu Thr Ile His Arg Phe Pro Gln Met  
 2005 2010 2015  
 Leu Glu Gly Ala Arg Gly Leu Val Asn Gln Leu Ile Gln Phe Gly Ser  
 2020 2025 2030  
 15 Ser Leu Leu Gly Tyr Ser Glu Arg Gln Asp Ala Glu Ala Met Ser Gln  
 2035 2040 2045  
 Leu Leu Gln Thr Gln Ala Ser Glu Leu Ile Leu Thr Ser Ile Arg Met  
 2050 2055 2060  
 Gln Asp Asn Gln Leu Ala Glu Leu Asp Ser Glu Lys Thr Ala Leu Gln  
 2065 2070 2075 2080  
 25 Val Ser Leu Ala Gly Val Gln Gln Arg Phe Asp Ser Tyr Ser Gln Leu  
 2085 2090 2095  
 Tyr Glu Glu Asn Ile Asn Ala Gly Glu Gln Arg Ala Leu Ala Leu Arg  
 2100 2105 2110  
 30 Ser Glu Ser Ala Ile Glu Ser Gln Gly Ala Gln Ile Ser Arg Met Ala  
 2115 2120 2125  
 Gly Ala Gly Val Asp Met Ala Pro Asn Ile Phe Gly Leu Ala Asp Gly  
 2130 2135 2140  
 Gly Met His Tyr Gly Ala Ile Ala Tyr Ala Ile Ala Asp Gly Ile Glu  
 2145 2150 2155 2160  
 40 Leu Ser Ala Ser Ala Lys Met Val Asp Ala Glu Lys Val Ala Gln Ser  
 2165 2170 2175  
 Glu Ile Tyr Arg Arg Arg Arg Gln Glu Trp Lys Ile Gln Arg Asp Asn  
 2180 2185 2190  
 45 Ala Gln Ala Glu Ile Asn Gln Leu Asn Ala Gln Leu Glu Ser Leu Ser  
 2195 2200 2205  
 Ile Arg Arg Glu Ala Ala Glu Met Gln Lys Glu Tyr Leu Lys Thr Gln  
 2210 2215 2220  
 Gln Ala Gln Ala Gln Ala Gln Leu Thr Phe Leu Arg Ser Lys Phe Ser  
 2225 2230 2235 2240  
 55 Asn Gln Ala Leu Tyr Ser Trp Leu Arg Gly Arg Leu Ser Gly Ile Tyr  
 2245 2250 2255  
 Phe Gln Phe Tyr Asp Leu Ala Val Ser Arg Cys Leu Met Ala Glu Gln  
 2260 2265 2270  
 60 Ser Tyr Gln Trp Glu Ala Asn Asp Asn Ser Ile Ser Phe Val Lys Pro  
 2275 2280 2285  
 Gly Ala Trp Gln Gly Thr Tyr Ala Gly Leu Leu Cys Gly Glu Ala Leu  
 2290 2295 2300

Ile Gln Asn Leu Ala Gln Met Glu Glu Ala Tyr Leu Lys Trp Glu Ser  
 2305 2310 2315 2320  
 5 Arg Ala Leu Glu Val Glu Arg Thr Val Ser Leu Ala Val Val Tyr Asp  
 2325 2330 2335  
 Ser Leu Glu Gly Asn Asp Arg Phe Asn Leu Ala Glu Gln Ile Pro Ala  
 2340 2345 2350  
 10 Leu Leu Asp Lys Gly Glu Gly Thr Ala Gly Thr Lys Glu Asn Gly Leu  
 2355 2360 2365  
 Ser Leu Ala Asn Ala Ile Leu Ser Ala Ser Val Lys Leu Ser Asp Leu  
 2370 2375 2380  
 15 Lys Leu Gly Thr Asp Tyr Pro Asp Ser Ile Val Gly Ser Asn Lys Val  
 2385 2390 2395 2400  
 20 Arg Arg Ile Lys Gln Ile Ser Val Ser Leu Pro Ala Leu Val Gly Pro  
 2405 2410 2415  
 Tyr Gln Asp Val Gln Ala Met Leu Ser Tyr Gly Gly Ser Thr Gln Leu  
 2420 2425 2430  
 25 Pro Lys Gly Cys Ser Ala Leu Ala Val Ser His Gly Thr Asn Asp Ser  
 2435 2440 2445  
 Gly Gln Phe Gln Leu Asp Phe Asn Asp Gly Lys Tyr Leu Pro Phe Glu  
 2450 2455 2460  
 30 Gly Ile Ala Leu Asp Asp Gln Gly Thr Leu Asn Leu Gln Phe Pro Asn  
 2465 2470 2475 2480  
 35 Ala Thr Asp Lys Gln Lys Ala Ile Leu Gln Thr Met Ser Asp Ile Ile  
 2485 2490 2495  
 Leu His Ile Arg Tyr Thr Ile Arg \*  
 2500 2505

40

## (2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 12 amino acids  
 45 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide  
 50

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

55 Leu Ile Gly Tyr Asn Asn Gln Phe Ser Gly Xaa Ala  
 1 5 10

## (2) INFORMATION FOR SEQ ID NO:14:

60 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 12 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Gln Asn Ser Gln Thr Phe Ser Val Gly Glu Leu  
1 5 10

10

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

15

(A) LENGTH: 9 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

25

Ala Gln Asp Gly Asn Gln Asp Thr Phe Phe Ser Gly Asn Thr  
1 5 10

(2) INFORMATION FOR SEQ ID NO:16:

30

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 5 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

40

Met Gln Asn Ser Leu  
1 5

45 (2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 10 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

50

(ii) MOLECULE TYPE: peptide

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Ala Phe Asn Ile Asp Asp Val Ser Leu Phe  
1 5 10

60



## (2) INFORMATION FOR SEQ ID NO:18:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 16 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

10

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

15 Phe Ile Val Tyr Thr Ser Leu Gly Val Asn Pro Asn Asn Ser Ser Asn  
1 5 10 15

## (2) INFORMATION FOR SEQ ID NO:19:

## (i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 21 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
25 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

30

Ile Ser Asp Leu Val Thr Thr Ser Pro Leu Ser Glu Ala Ile Gly Ser  
1 5 10 15

35 Leu Gln Leu Phe Ile  
20

## (2) INFORMATION FOR SEQ ID NO:20:

## (i) SEQUENCE CHARACTERISTICS:

- 40 (A) LENGTH: 12 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
45 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

50

Met Tyr Tyr Ile Gln Ala Gln Gln Leu Leu Gly Pro  
1 5 10

## 55 (2) INFORMATION FOR SEQ ID NO:21:

## (i) SEQUENCE CHARACTERISTICS:

- 60 (A) LENGTH: 26 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Gly Ile Asp Ala Val Leu Ser Met Glu Thr Gln Asn Ile Gln Glu Pro  
1 5 10 15

10 Gln Leu Gly Ala Gly Thr Tyr Val Gln Leu  
20 25

(2) INFORMATION FOR SEQ ID NO:22:

15

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 15 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: peptide

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Ile Ser Asn Pro Ile Asn Ile Asn Thr Gly Ile Asp Ser Ala Lys  
1 5 10 15

30

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 13 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: peptide

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Thr Tyr Leu Thr Ser Phe Glu Gln Val Ala Asn Leu Lys  
1 5 10

45

(2) INFORMATION FOR SEQ ID NO:24:

- 50 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 22 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

55

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

60

Val Leu Gly Thr Glu Asn Val Ile Ala Leu Tyr Ser Glu Asn Asn Gly

1 5 10 15  
 Val Gln Tyr Met Gln Ile  
 20

5

## (2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 6005 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (ix) FEATURE:  
 (A) NAME/KEY: RBS  
 (B) LOCATION: 1..9
- (ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 16..3585  
 (D) OTHER INFORMATION: /product= "P8"

25

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

AAGAAGGAAT TGATT ATG TCT GAA TCT TTA TTT ACA CAA ACG TTG AAA GAA 51  
 Met Ser Glu Ser Leu Phe Thr Gln Thr Leu Lys Glu  
 1 5 10

GCG CGC CGT GAT GCA TTG GTT GCT CAT TAT ATT GCT ACT CAG GTG CCC 99  
 Ala Arg Arg Asp Ala Leu Val Ala His Tyr Ile Ala Thr Gln Val Pro  
 15 20 25

GCA GAT TTA AAA GAG AGT ATC CAG ACC GCG GAT GAT CTG TAC GAA TAT 147  
 Ala Asp Leu Lys Glu Ser Ile Gln Thr Ala Asp Asp Leu Tyr Glu Tyr  
 30 35 40

CTG TTG CTG GAT ACC AAA ATT AGC GAT CTG GTT ACT ACT TCA CCG CTG 195  
 Leu Leu Leu Asp Thr Lys Ile Ser Asp Leu Val Thr Thr Ser Pro Leu  
 45 50 55 60

TCC GAA GCG ATT GGC AGT CTG CAA TTG TTT ATT CAT CGT GCG ATA GAG 243  
 Ser Glu Ala Ile Gly Ser Leu Gln Leu Phe Ile His Arg Ala Ile Glu  
 65 70 75

GGC TAT GAC GGC ACG CTG GCA GAC TCA GCA AAA CCC TAT TTT GCC GAT 291  
 Gly Tyr Asp Gly Thr Leu Ala Asp Ser Ala Lys Pro Tyr Phe Ala Asp  
 80 85 90

GAA CAG TTT TTA TAT AAC TGG GAT AGT TTT AAC CAC CGT TAT AGC ACT 339  
 Glu Gln Phe Leu Tyr Asn Trp Asp Ser Phe Asn His Arg Tyr Ser Thr  
 95 100 105

TGG GCT GGC AAG GAA CGG TTG AAA TTC TAT GCC GGG GAT TAT ATT GAT 387  
 Trp Ala Gly Lys Glu Arg Leu Lys Phe Tyr Ala Gly Asp Tyr Ile Asp  
 110 115 120

CCA ACA TTG CGA TTG AAT AAG ACC GAG ATA TTT ACC GCA TTT GAA CAA 435  
 Pro Thr Leu Arg Leu Asn Lys Thr Glu Ile Phe Thr Ala Phe Glu Gln  
 125 130 135 140

	GGT ATT TCT CAA GGG AAA TTA AAA AGT GAA TTA GTC GAA TCT AAA TTA 433
	Gly Ile Ser Gln Gly Lys Leu Lys Ser Glu Leu Val Glu Ser Lys Leu 155
5	CGT GAT TAT CTA ATT AGT TAT GAC ACT TTA GCC ACC CTT GAT TAT ATT 531
	Arg Asp Tyr Leu Ile Ser Tyr Asp Thr Leu Ala Thr Leu Asp Tyr Ile 170
10	ACT GCC TGC CAA GGC AAA GAT AAT AAA ACC ATC TTC TTT ATT GGC CGT 579
	Thr Ala Cys Gln Gly Lys Asp Asn Lys Thr Ile Phe Phe Ile Gly Arg 185
15	ACA CAG AAT GCA CCC TAT GCA TTT TAT TGG CGA AAA TTA ACT TTA GTC 627
	Thr Gln Asn Ala Pro Tyr Ala Phe Tyr Trp Arg Lys Leu Thr Leu Val 200
20	ACT GAT GGC GGT AAG TTG AAA CCA GAT CAA TGG TCA GAG TGG CGA GCA 675
	Thr Asp Gly Gly Lys Leu Lys Pro Asp Gln Trp Ser Glu Trp Arg Ala 220
	ATT AAT GCC GGG ATT AGT GAG GCA TAT TGA GGG CAT GTC GAG CCT TTC 723
	Ile Asn Ala Gly Ile Ser Glu Ala Tyr Ser Gly His Val Glu Pro Phe 235
25	TGG GAA AAT AAC AAG CTG CAC ATC CGT TGG TTT ACT ATC TCG AAA GAA 771
	Trp Glu Asn Asn Lys Leu His Ile Arg Trp Phe Thr Ile Ser Lys Glu 250
30	GAT AAA ATA GAT TTT GTT TAT AAA AAC ATC TGG GTG ATG AGT AGC GAT 819
	Asp Lys Ile Asp Phe Val Tyr Lys Asn Ile Trp Val Met Ser Ser Asp 265
35	TAT AGC TGG GCA TCA AAG AAA AAA ATC TTG GAA CTT TCT TTT ACT GAC 867
	Tyr Ser Trp Ala Ser Lys Lys Lys Ile Leu Glu Leu Ser Phe Thr Asp 280
40	TAC AAT AGA GTT GGA GCA ACA GGA TCA TCA AGC CCG ACT GAA GTA GCT 915
	Tyr Asn Arg Val Gly Ala Thr Gly Ser Ser Ser Pro Thr Glu Val Ala 300
	TCA CAA TAT GGT TCT GAT GCT CAG ATG AAT ATT TCT GAT GAT GGG ACT 963
	Ser Gln Tyr Gly Ser Asp Ala Gln Met Asn Ile Ser Asp Asp Gly Thr 315
45	GTA CTT ATT TTT CAG AAT GCC GGC GGA GCT ACT CCC AGT ACT GGA GTG 1011
	Val Leu Ile Phe Gln Asn Ala Gly Gly Ala Thr Pro Ser Thr Gly Val 330
50	ACG TTA TGT TAT GAC TCT GGC AAC GTG ATT AAG AAC CTA TCT AGT ACA 1059
	Thr Leu Cys Tyr Asp Ser Gly Asn Val Ile Lys Asn Leu Ser Ser Thr 345
55	GGA AGT GCA AAT TTA TCG TCA AAG GAT TAT GCC ACA ACT AAA TTA CGC 1107
	Gly Ser Ala Asn Leu Ser Ser Lys Asp Tyr Ala Thr Thr Lys Leu Arg 360
60	ATG TGT CAT GGA CAA AGT TAC AAT GAT AAT AAC TAC TGC AAT TTT ACA 1155
	Met Cys His Gly Gln Ser Tyr Asn Asp Asn Asn Tyr Cys Asn Phe Thr 380
	CTC TCT ATT AAT ACA ATA GAA TTC ACC TCC TAC GGC ACA TTC TCA TCA 1203
	Leu Ser Ile Asn Thr Ile Glu Phe Thr Ser Tyr Gly Thr Phe Ser Ser 395
65	GAT GGA AAA CAA TTT ACA CCA CCT TCT GGT TCT GCC ATT GAT TTA CAC 1251
	Asp Gly Lys Gln Phe Thr Pro Pr Ser Gly Ser Ala Ile Asp Leu His

	400										405										410										
5	CTC	CCT	AAT	TAT	GTA	GAT	CTC	AAC	GCG	CTA	TTA	GAT	ATT	AGC	CTC	GAT	1299														
	Leu	Pro	Asn	Tyr	Val	Asp	Leu	Asn	Ala	Leu	Leu	Asp	Ile	Ser	Leu	Asp															
			415					420					425																		
10	TCA	CTA	CTT	AAT	TAT	GAC	GTT	CAG	GGG	CAG	TTT	GGC	GGA	TCT	AAT	CCG	1347														
	Ser	Leu	Leu	Asn	Tyr	Asp	Val	Gln	Gly	Gln	Phe	Gly	Gly	Ser	Asn	Pro															
			430				435					440																			
15	GTT	GAT	AAT	TTC	AGT	GGT	CCC	TAT	GGT	ATT	TAT	CTA	TGG	GAA	ATC	TTC	1395														
	Val	Asp	Asn	Phe	Ser	Gly	Pro	Tyr	Gly	Ile	Tyr	Leu	Trp	Glu	Ile	Phe															
			445			450					455					460															
20	TTC	CAT	ATT	CCG	TTC	CTT	GTT	ACG	GTC	CGT	ATG	CAA	ACC	GAA	CAA	CGT	1443														
	Phe	His	Ile	Pro	Phe	Leu	Val	Thr	Val	Arg	Met	Gln	Thr	Glu	Gln	Arg															
					465				470						475																
25	TAC	GAA	GAC	GCG	GAC	ACT	TGG	TAC	AAA	TAT	ATT	TTC	CGC	AGC	GCC	GGT	1491														
	Tyr	Glu	Asp	Ala	Asp	Thr	Trp	Tyr	Lys	Tyr	Ile	Phe	Arg	Ser	Ala	Gly															
				480					485					490																	
30	TAT	CGC	GAT	GCT	AAT	GGC	CAG	CTC	ATT	ATG	GAT	GGC	AGT	AAA	CCA	CGT	1539														
	Tyr	Arg	Asp	Ala	Asn	Gly	Gln	Leu	Ile	Met	Asp	Gly	Ser	Lys	Pro	Arg															
			495				500						505																		
35	TAT	TGG	AAT	GTG	ATG	CCA	TTG	CAA	CTG	GAT	ACC	GCA	TGG	GAT	ACC	ACA	1587														
	Tyr	Trp	Asn	Val	Met	Pro	Leu	Gln	Leu	Asp	Thr	Ala	Trp	Asp	Thr	Thr															
			510				515					520																			
40	CAG	CCC	GCC	ACC	ACT	GAT	CCA	GAT	GTG	ATC	GCT	ATG	GCG	GAC	CCG	ATG	1635														
	Gln	Pro	Ala	Thr	Thr	Asp	Pro	Asp	Val	Ile	Ala	Met	Ala	Asp	Pro	Met															
			525			530				535					540																
45	CAT	TAC	AAG	CTG	GCG	ATA	TTC	CTG	CAT	ACC	CTT	GAT	CTA	TTG	ATT	GCC	1683														
	His	Tyr	Lys	Leu	Ala	Ile	Phe	Leu	His	Thr	Leu	Asp	Leu	Leu	Ile	Ala															
				545					550					555																	
50	CGA	GGC	GAC	AGC	GCT	TAC	CGT	CAA	CTT	GAA	CGC	GAT	ACT	CTA	GTC	GAA	1731														
	Arg	Gly	Asp	Ser	Ala	Tyr	Arg	Gln	Leu	Glu	Arg	Asp	Thr	Leu	Val	Glu															
				560					565					570																	
55	GCC	AAA	ATG	TAC	TAC	ATT	CAG	GCA	CAA	CAG	CTA	CTG	GGA	CCG	CGC	CCT	1779														
	Ala	Lys	Met	Tyr	Tyr	Ile	Gln	Ala	Gln	Gln	Leu	Leu	Gly	Pro	Arg	Pro															
			575				580						585																		
60	GAT	ATC	CAT	ACC	ACC	AAT	ACT	TGG	CCA	AAT	CCC	ACC	TTG	AGT	AAA	GAA	1827														
	Asp	Ile	His	Thr	Thr	Asn	Thr	Trp	Pro	Asn	Pro	Thr	Leu	Ser	Lys	Glu															
			590				595					600																			
65	GCT	GGC	GCT	ATT	GCC	ACA	CCG	ACA	TTC	CTC	AGT	TCA	CCG	GAG	GTG	ATG	1875														
	Ala	Gly	Ala	Ile	Ala	Thr	Pro	Thr	Phe	Leu	Ser	Ser	Pro	Glu	Val	Met															
			605			610				615					620																
70	ACG	TTC	GCT	GCC	TGG	CTA	AGC	GCA	GGC	GAT	ACC	GCA	AAT	ATT	GGC	GAC	1923														
	Thr	Phe	Ala	Ala	Trp	Leu	Ser	Ala	Gly	Asp	Thr	Ala	Asn	Ile	Gly	Asp															
				625					630					635																	
75	GGT	GAT	TTC	TTG	CCA	CCG	TAC	AAC	GAT	GTA	CTA	CTC	GGT	TAC	TGG	GAT	1971														
	Gly	Asp	Phe	Leu	Pro	Pro	Tyr	Asn	Asp	Val	Leu	Leu	Gly	Tyr	Trp	Asp															
				640					645					650																	
80	AAA	CTT	GAG	TTA	CGC	CTA	TAC	AAC	CTG	CGC	CAC	AAT	CTG	AGT	CTG	GAT	2019														
	Lys	Leu	Glu	Leu	Arg	Leu	Tyr	Asn	Leu	Arg	His	Asn	Leu	Ser	Leu	Asp															
			655				660						665																		

	3GT CAA CCG CTA AAT CTG CCA CTG TAT GCC ACG CCG GTA GAC CCG AAA 2057	
	Gly Gln Pro Leu Asn Leu Pro Leu Tyr Ala Thr Pro Val Asp Pro Lys	
	670 675 680	
5	ACC CTG CAA CGC CAG CAA GCC GGA GGG GAC GGT ACA GGC AGT AGT CCG 2115	
	Thr Leu Gln Arg Gln Ala Gly Gly Asp Thr Gly Ser Ser Pro	
	685 690 695 700	
10	GCT GGT GGT CAA GGC AGT GTT CAG GGC TGG CCG TAT CCG TTA TTG GTA 2153	
	Ala Gly Gly Gln Gly Ser Val Gln Gly Trp Arg Tyr Pro Leu Leu Val	
	705 710 715	
15	GAA CCG GCC CGC TCT GCC GTG AGT TTG TTG ACT CAG TTC GGC AAC AGC 2211	
	Glu Arg Ala Arg Ser Ala Val Ser Leu Leu Thr Gln Phe Gly Asn Ser	
	720 725 730	
20	TTA CAA ACA ACG TTA GAA CAT CAG GAT AAT GAA AAA ATG ACG ATA CTG 2259	
	Leu Gln Thr Thr Leu Glu His Gln Asp Asn Glu Lys Met Thr Ile Leu	
	735 740 745	
25	TTG CAG ACT CAA CAG GAA GCC ATC CTG AAA CAT CAG CAC GAT ATA CAA 2307	
	Leu Gln Thr Gln Gln Glu Ala Ile Leu Lys His Gln His Asp Ile Gln	
	750 755 760	
30	CAA AAT AAT CTA AAA GGA TTA CAA CAC AGC CTG ACC GCA TTA CAG GCT 2355	
	Gln Asn Asn Leu Lys Gly Leu Gln His Ser Leu Thr Ala Leu Gln Ala	
	765 770 775 780	
35	AGC CGT GAT GGC GAC ACA TTG CGG CAA AAA CAT TAC AGC GAC CTG ATT 2403	
	Ser Arg Asp Gly Asp Thr Leu Arg Gln Lys His Tyr Ser Asp Leu Ile	
	785 790 795	
40	AAC GGT GGT CTA TCT GCG GCA GAA ATC GCC GGT CTG ACA CTA CGC AGC 2451	
	Asn Gly Gly Leu Ser Ala Ala Glu Ile Ala Gly Leu Thr Leu Arg Ser	
	800 805 810	
45	ACC GCC ATG ATT ACC AAT GGC GTT GCA ACG GGA TTG CTG ATT GCC GGC 2499	
	Thr Ala Met Ile Thr Asn Gly Val Ala Thr Gly Leu Leu Ile Ala Gly	
	815 820 825	
50	GGA ATC GCC AAC GCG GTA CCT AAC GTC TTC GGG CTG GCT AAC GGT GGA 2547	
	Gly Ile Ala Asn Ala Val Pro Asn Val Phe Gly Leu Ala Asn Gly Gly	
	830 835 840	
55	TCG GAA TGG GGA GCG CCA TTA ATT GGC TCC GGG CAA GCA ACC CAA GTT 2595	
	Ser Glu Trp Gly Ala Pro Leu Ile Gly Ser Gly Gln Ala Thr Gln Val	
	845 850 855 860	
60	GGC GCC GGC ATC CAG GAT CAG AGC GCG GGC ATT TCA GAA GTG ACA GCA 2643	
	Gly Ala Gly Ile Gln Asp Gln Ser Ala Gly Ile Ser Glu Val Thr Ala	
	865 870 875	
65	GGC TAT CAG CGT CGT CAG GAA GAA TGG GCA TTG CAA CGG GAT ATT GCT 2691	
	Gly Tyr Gln Arg Arg Gln Glu Glu Trp Ala Leu Gln Arg Asp Ile Ala	
	880 885 890	
70	GAT AAC GAA ATA ACC CAA CTG GAT GCC CAG ATA CAA AGC CTG CAA GAG 2739	
	Asp Asn Glu Ile Thr Gln Leu Asp Ala Gln Ile Gln Ser Leu Gln Glu	
	895 900 905	
75	CAA ATC ACG ATG GCA CAA AAA CAG ATC ACG CTC TCT GAA ACC GAA CAA 2787	
	Gln Ile Thr Met Ala Gln Lys Gln Ile Thr Leu Ser Glu Thr Glu Gln	
	910 915 920	
80	GCG AAT GCC CAA GCG ATT TAT GAC CTG CAA ACC ACT CGT TTT ACC GGG 2835	
	Ala Asn Ala Gln Ala Ile Tyr Asp Leu Gln Thr Thr Arg Phe Thr Gly	

	925		930		935		940	
5	CAG GCA CTG TAT AAC TGG ATG GCC GGT CGT CTC TCC GCG CTC TAT TAC 2333 Gln Ala Leu Tyr Asn Trp Met Ala Gly Arg Leu Ser Ala Leu Tyr Tyr 955							
10	CAA ATG TAT GAT TCC ACT CTG CCA ATC TGT CTC CAG CCA AAA GCC GCA 2931 Gln Met Tyr Asp Ser Thr Leu Pro Ile Cys Leu Gln Pro Lys Ala Ala 970							
15	TTA GTA CAG GAA TTA GGC GAG AAA GAG AGC GAC AGT CTT TTC CAG GTT 2979 Leu Val Gln Glu Leu Gly Glu Lys Glu Ser Asp Ser Leu Phe Gln Val 985							
20	CCG GTG TGG AAT GAT CTG TGG CAA GGG CTG TTA GCA GGA GAA GGT TTA 3027 Pro Val Trp Asn Asp Leu Trp Gln Gly Leu Leu Ala Gly Glu Gly Leu 990 1000							
25	AGT TCA GAG CTA CAG AAA CTG GAT GCC ATC TGG CTT GCA CGT GGT GGT 3075 Ser Ser Glu Leu Gln Lys Leu Asp Ala Ile Trp Leu Ala Arg Gly Gly 1005 1010 1015 1020							
30	ATT GGG CTA GAA GCC ATC CGC ACC GTG TCG CTG GAT ACC CTG TTT GGC 3123 Ile Gly Leu Glu Ala Ile Arg Thr Val Ser Leu Asp Thr Leu Phe Gly 1025 1030 1035							
35	ACA GGG ACG TTA AGT GAA AAT ATC AAT AAA GTG CTT AAC GGG GAA ACG 3171 Thr Gly Thr Leu Ser Glu Asn Ile Asn Lys Val Leu Asn Gly Glu Thr 1040 1045 1050							
40	GTA TCT CCA TCC GGT GGC GTC ACT CTG GCG CTG ACA GGG GAT ATC TTC 3219 Val Ser Pro Ser Gly Gly Val Thr Leu Ala Leu Thr Gly Asp Ile Phe 1055 1060 1065							
45	CAA GCA ACA CTG GAT TTG AGT CAG CTA GGT TTG GAT AAC TCT TAC AAC 3267 Gln Ala Thr Leu Asp Leu Ser Gln Leu Gly Leu Asp Asn Ser Tyr Asn 1070 1075 1080							
50	TTG GGT AAC GAG AAG AAA CGT CGT ATT AAA CGT ATC GCC GTC ACC CTG 3315 Leu Gly Asn Glu Lys Lys Arg Arg Ile Lys Arg Ile Ala Val Thr Leu 1085 1090 1095 1100							
55	CCA ACA CTT CTG GGG CCA TAT CAA GAT CTT GAA GCC ACA CTG GTA ATG 3363 Pro Thr Leu Leu Gly Pro Tyr Gln Asp Leu Glu Ala Thr Leu Val Met 1105 1110 1115							
60	GGT GCG GAA ATC GCC GCC TTA TCA CAC GGT GTG AAT GAC GGA GGC CGG 3411 Gly Ala Glu Ile Ala Ala Leu Ser His Gly Val Asn Asp Gly Gly Arg 1120 1125 1130							
65	TTT GTT ACC GAC TTT AAC GAC AGC CGT TTT CTG CCT TTT GAA GGT CGA 3459 Phe Val Thr Asp Phe Asn Asp Ser Arg Phe Leu Pro Phe Glu Gly Arg 1135 1140 1145							
70	GAT GCA ACA ACC GGC ACA CTG GAG CTC AAT ATT TTC CAT GCG GGT AAA 3507 Asp Ala Thr Thr Gly Thr Leu Glu Leu Asn Ile Phe His Ala Gly Lys 1150 1155 1160							
75	GAG GGA ACG CAA CAC GAG TTG GTC GCG AAT CTG AGT GAC ATC ATT GTG 3555 Glu Gly Thr Gln His Glu Leu Val Ala Asn Leu Ser Asp Ile Ile Val 1165 1170 1175 1180							
80	CAT CTG AAT TAC ATC ATT CGA GAC GCG TAA ATTTCTTTTC TTGTGCGATT 3605 His Leu Asn Tyr Ile Ile Arg Asp Ala * 1185 1190							

ACAGGTCCCT ATCAGGGGCC TGTATTAAAG GAGTACTTTA TGCAGGATTC ACCAGAAGTA 3665  
TCGATTACAA CGCTGTCACT TCCCAAAGGT GCGGGTGCTA TCAATGGCAT GGGAGAAGCA 3725  
5 CTGAATGCTG CCGGCCCTGA TGGAAATGGCC TCCCTATCTC TGCCATTACC CCTTTGACC 3785  
GGCAAGAGGA CGGCTCCTGG ATTATCGCTG ATTTACAGCA ACAGTGCAGG TAATGGGCCT 3845  
10 TTCGGCATCG GCTGGCAATG CGGTGTTATG TCCATTAGCC GACGCACCCA ACATGGCATT 3905  
CCACAATACG GTAATGACGA CACGTTCTTA TCCCCACAAG GCGAGGTCAT GAATATCGCC 3965  
CTGAATGACC AAGGGCAACC TGATATCCGT CAAGACGTTA AAACGCTGCA AGGCGTTACC 4025  
15 TTGCCAATTT CCTATACCGT GACCCGCTAT CAAGCCCGCC AGATCCTGGA TTTCAGTAA 4085  
ATCGAATACT GGCAACCTGC CTCCGGTCAA GAAGGACGCG CTTTCTGGCT GATATCGACA 4145  
CCGGACGGGC ATCTACACAT CTTAGGGAAA ACCGCGCAGG CTTGTCTGGC AAATCCGCAA 4205  
20 AATGACCAAC AAATCGCCCA GTGGTTGCTG GAAGAACTG TGACGCCAGC CGGTGAACAT 4265  
GTCAGCTATC AATATCGAGC CGAAGATGAA GCCCATGTG ACGACAATGA AAAAACCCT 4325  
25 CATCCCAATG TTACCGCACA GCGCTATCTG GTACAGGTGA ACTACAGGCA ACATCAAACC 4385  
ACAAGCCAGC CTGTTCTGTAC TGGATAACGC ACCTCCCGCA CCGGAAGAGT GGCTGTTTCA 4445  
TCTGGTCTTT GACCACGGTG AGCGCGTACC TCACCTCATA CCGTGCCAAC ATGGGATGCA 4505  
30 GGTACAGCGC AATGGTCTGT ACGCCCGGAT ATCTTCTCTC GCTATGAATA TGGTTTTGAA 4565  
GTGCGTACTC GCCGCTTATG TCAACAAGTG CTGATGTTTC ACCGCACCGC GCTCATGGCC 4625  
35 GGAGAAGCCA GTACCAATGA CGCCCCGAA CTGGTTGGAC GCTTAATACT GGAATATGAC 4685  
AAAAACGCCA GCGTCACCAC GTTGATTACC ATCCGTCAAT TAAGCCATGA ATCGGACGGG 4745  
AGGCCAGTCA CCCAGCCACC ACTAGAACTA GCCTGGCAAC GGTGTGATCT GGAGAAAATC 4805  
40 CCGACATGGC AACGCTTTGA CGCACTAGAT AATTTTAACT CGCAGCAACG TTATCAACTG 4865  
GTTGATCTGC GGGGAGAAGG GTTGCCAGGT ATGCTGTATC AAGATCGAGG CGCTTGGTGG 4925  
45 TATAAAGCTC CGCAACGTCA GGAAGACGGA GACAGCAATG CCGTCACTTA CGACAAAATC 4985  
GCCCCACTGC CTACCCTACC CAATTTGCAG GATAATGCCT CATTGATGGA TATCAACGGA 5045  
GACGGCCAAC TGGATTGGGT TGTACCGCC TCCGGTATTC GCGGATACCA TAGTCAGCAA 5105  
50 CCCGATGGAA AGTGGACGCA CTTTACGCCA ATCAATGCCT TGCCCGTGGA ATATTTTCAT 5165  
CCAAGCATCC AGTTCGCTGA CCTTACCGGG GCAGGCTTAT CTGATTTAGT GTTGATCGGG 5225  
55 CCGAAAAGCG TCGTCTATA TGCCAACCAG CGAAACGGCT GCGGTAAAGG AGAAGATGTC 5285  
CCCCAATCCA CAGGTATCAC CCTGCCTGTC ACAGGGACCG ATGCCCGCAA ACTGGTGGCT 5345  
TTCAGTGATA TGCTCGGTTT CCGTCAACAA CATCTGGTGG AAATCAAGGG TAATCGCGTC 5405  
60 ACCTGTTGGC CGAATCTAGG GCATGGCCGT TTCGGTCAAC CACTAACTCT GTCAGGATTT 5465  
AGCCAGCCCG AAAATAGCTT CAATCCCGAA CGGCTGTTTC TGGCGGATAT CGACGGCTCC 5525  
65 GGCACCACCG ACCTTATCTA TCGCAATCC GGCTCTTTGC TCATTTATCT CAACCAAAGT 5585



5 GTTAATCAGT TTGATGCCCC GTTGACATTA GCGTTGCCAG AAGGCGTACA ATTTGACAAC 5645  
 ACTTGCCAAC TTCAAGTCGC CGATATTCAG GGATTAGGGA TAGCCAGCTT GATTCTGACT 5705  
 10 GTGCCACATA TCGCGCCACA TCACTGGCGT TGTGACCTGT CACTGACCAA ACCCTGGTTG 5765  
 TTGAATGTAA TGAACAATAA CCGGGGCGCA CATCACACGC TACATTATCG TAGTTCCGCG 5825  
 CAATTCTGGT TGGATGAAAA ATTACAGCTC ACCAAAGCAG GCAAATCTCC GGCTTGTTAT 5885  
 15 CTGCCGTTTC CAATGCATTT GCTATGGTAT ACCGAAATTC AGGATGAAAT CAGCGGCAAC 5945  
 CGGCTCACCA GTGAAGTCAA CTACAGCCAC GCGCTCTGGG ATGGTAAAGA GCGGGAATTC 6005

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1190 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Met Ser Glu Ser Leu Phe Thr Gln Thr Leu Lys Glu Ala Arg Arg Asp  
 1 5 10 15  
 30 Ala Leu Val Ala His Tyr Ile Ala Thr Gln Val Pro Ala Asp Leu Lys  
 20 25 30  
 35 Glu Ser Ile Gln Thr Ala Asp Asp Leu Tyr Glu Tyr Leu Leu Leu Asp  
 35 40 45  
 Thr Lys Ile Ser Asp Leu Val Thr Thr Ser Pro Leu Ser Glu Ala Ile  
 50 55 60  
 40 Gly Ser Leu Gln Leu Phe Ile His Arg Ala Ile Glu Gly Tyr Asp Gly  
 65 70 75 80  
 Thr Leu Ala Asp Ser Ala Lys Pro Tyr Phe Ala Asp Glu Gln Phe Leu  
 85 90 95  
 45 Tyr Asn Trp Asp Ser Phe Asn His Arg Tyr Ser Thr Trp Ala Gly Lys  
 100 105 110  
 50 Glu Arg Leu Lys Phe Tyr Ala Gly Asp Tyr Ile Asp Pro Thr Leu Arg  
 115 120 125  
 Leu Asn Lys Thr Glu Ile Phe Thr Ala Phe Glu Gln Gly Ile Ser Gln  
 130 135 140  
 55 Gly Lys Leu Lys Ser Glu Leu Val Glu Ser Lys Leu Arg Asp Tyr Leu  
 145 150 155 160  
 Ile Ser Tyr Asp Thr Leu Ala Thr Leu Asp Tyr Ile Thr Ala Cys Gln  
 165 170 175  
 60 Gly Lys Asp Asn Lys Thr Ile Phe Phe Ile Gly Arg Thr Gln Asn Ala  
 180 185 190  
 65 Pro Tyr Ala Phe Tyr Trp Arg Lys Leu Thr Leu Val Thr Asp Gly Gly  
 195 200 205

	Lys	Leu	Lys	Pro	Asp	Gln	Trp	Ser	Glu	Trp	Arg	Ala	Ile	Asn	Ala	Gly	
	210						215					220					
5	Ile	Ser	Glu	Ala	Tyr	Ser	Gly	His	Val	Glu	Pro	Phe	Trp	Glu	Asn	Asn	
	225					230					235					240	
	Lys	Leu	His	Ile	Arg	Trp	Phe	Thr	Ile	Ser	Lys	Glu	Asp	Lys	Ile	Asp	
					245					250					255		
10	Phe	Val	Tyr	Lys	Asn	Ile	Trp	Val	Met	Ser	Ser	Asp	Tyr	Ser	Trp	Ala	
				260					265					270			
	Ser	Lys	Lys	Lys	Ile	Leu	Glu	Leu	Ser	Phe	Thr	Asp	Tyr	Asn	Arg	Val	
15			275					280					285				
	Gly	Ala	Thr	Gly	Ser	Ser	Ser	Pro	Thr	Glu	Val	Ala	Ser	Gln	Tyr	Gly	
	290						295					300					
20	Ser	Asp	Ala	Gln	Met	Asn	Ile	Ser	Asp	Asp	Gly	Thr	Val	Leu	Ile	Phe	
	305					310					315					320	
	Gln	Asn	Ala	Gly	Gly	Ala	Thr	Pro	Ser	Thr	Gly	Val	Thr	Leu	Cys	Tyr	
				325						330					335		
25	Asp	Ser	Gly	Asn	Val	Ile	Lys	Asn	Leu	Ser	Ser	Thr	Gly	Ser	Ala	Asn	
				340					345					350			
	Leu	Ser	Ser	Lys	Asp	Tyr	Ala	Thr	Thr	Lys	Leu	Arg	Met	Cys	His	Gly	
30			355					360					365				
	Gln	Ser	Tyr	Asn	Asp	Asn	Asn	Tyr	Cys	Asn	Phe	Thr	Leu	Ser	Ile	Asn	
	370					375						380					
35	Thr	Ile	Glu	Phe	Thr	Ser	Tyr	Gly	Thr	Phe	Ser	Ser	Asp	Gly	Lys	Gln	
	385					390					395					400	
	Phe	Thr	Pro	Pro	Ser	Gly	Ser	Ala	Ile	Asp	Leu	His	Leu	Pro	Asn	Tyr	
					405					410					415		
40	Val	Asp	Leu	Asn	Ala	Leu	Leu	Asp	Ile	Ser	Leu	Asp	Ser	Leu	Leu	Asn	
				420				425						430			
	Tyr	Asp	Val	Gln	Gly	Gln	Phe	Gly	Gly	Ser	Asn	Pro	Val	Asp	Asn	Phe	
45			435					440					445				
	Ser	Gly	Pro	Tyr	Gly	Ile	Tyr	Leu	Trp	Glu	Ile	Phe	Phe	His	Ile	Pro	
	450					455						460					
50	Phe	Leu	Val	Thr	Val	Arg	Met	Gln	Thr	Glu	Gln	Arg	Tyr	Glu	Asp	Ala	
	465					470					475					480	
	Asp	Thr	Trp	Tyr	Lys	Tyr	Ile	Phe	Arg	Ser	Ala	Gly	Tyr	Arg	Asp	Ala	
				485						490					495		
55	Asn	Gly	Gln	Leu	Ile	Met	Asp	Gly	Ser	Lys	Pro	Arg	Tyr	Trp	Asn	Val	
			500					505						510			
	Met	Pro	Leu	Gln	Leu	Asp	Thr	Ala	Trp	Asp	Thr	Thr	Gln	Pro	Ala	Thr	
60			515					520					525				
	Thr	Asp	Pro	Asp	Val	Ile	Ala	Met	Ala	Asp	Pro	Met	His	Tyr	Lys	Leu	
	530						535					540					
65	Ala	Ile	Phe	Leu	His	Thr	Leu	Asp	Leu	Leu	Ile	Ala	Arg	Gly	Asp	Ser	
	545					550					555					560	

	Ala Tyr Arg Gln Leu Glu Arg Asp Thr Leu Val Glu Ala Lys Met Tyr	565	570	575
5	Tyr Ile Gln Ala Gln Gln Leu Leu Gly Pro Arg Pro Asp Ile His Thr	580	585	590
	Thr Asn Thr Trp Pro Asn Pro Thr Leu Ser Lys Glu Ala Gly Ala Ile	595	600	605
10	Ala Thr Pro Thr Phe Leu Ser Ser Pro Glu Val Met Thr Phe Ala Ala	610	615	620
	Trp Leu Ser Ala Gly Asp Thr Ala Asn Ile Gly Asp Gly Asp Phe Leu	625	630	635
15	Pro Pro Tyr Asn Asp Val Leu Leu Gly Tyr Trp Asp Lys Leu Glu Leu	645	650	655
	Arg Leu Tyr Asn Leu Arg His Asn Leu Ser Leu Asp Gly Gln Pro Leu	660	665	670
	Asn Leu Pro Leu Tyr Ala Thr Pro Val Asp Pro Lys Thr Leu Gln Arg	675	680	685
25	Gln Gln Ala Gly Gly Asp Gly Thr Gly Ser Ser Pro Ala Gly Gly Gln	690	695	700
	Gly Ser Val Gln Gly Trp Arg Tyr Pro Leu Leu Val Glu Arg Ala Arg	705	710	715
30	Ser Ala Val Ser Leu Leu Thr Gln Phe Gly Asn Ser Leu Gln Thr Thr	725	730	735
	Leu Glu His Gln Asp Asn Glu Lys Met Thr Ile Leu Leu Gln Thr Gln	740	745	750
	Gln Glu Ala Ile Leu Lys His Gln His Asp Ile Gln Gln Asn Asn Leu	755	760	765
40	Lys Gly Leu Gln His Ser Leu Thr Ala Leu Gln Ala Ser Arg Asp Gly	770	775	780
	Asp Thr Leu Arg Gln Lys His Tyr Ser Asp Leu Ile Asn Gly Gly Leu	785	790	795
45	Ser Ala Ala Glu Ile Ala Gly Leu Thr Leu Arg Ser Thr Ala Met Ile	805	810	815
	Thr Asn Gly Val Ala Thr Gly Leu Leu Ile Ala Gly Gly Ile Ala Asn	820	825	830
	Ala Val Pro Asn Val Phe Gly Leu Ala Asn Gly Gly Ser Glu Trp Gly	835	840	845
55	Ala Pro Leu Ile Gly Ser Gly Gln Ala Thr Gln Val Gly Ala Gly Ile	850	855	860
	Gln Asp Gln Ser Ala Gly Ile Ser Glu Val Thr Ala Gly Tyr Gln Arg	865	870	875
60	Arg Gln Glu Glu Trp Ala Leu Gln Arg Asp Ile Ala Asp Asn Glu Ile	885	890	895
	Thr Gln Leu Asp Ala Gln Ile Gln Ser Leu Gln Glu Gln Ile Thr Met	900	905	910

Ala Gln Lys Gln Ile Thr Leu Ser Glu Thr Glu Gln Ala Asn Ala Gln  
 915 920 925  
 5 Ala Ile Tyr Asp Leu Gln Thr Thr Arg Phe Thr Gly Gln Ala Leu Tyr  
 930 935 940  
 Asn Trp Met Ala Gly Arg Leu Ser Ala Leu Tyr Tyr Gln Met Tyr Asp  
 945 950 955 960  
 10 Ser Thr Leu Pro Ile Cys Leu Gln Pro Lys Ala Ala Leu Val Gln Glu  
 965 970 975  
 Leu Gly Glu Lys Glu Ser Asp Ser Leu Phe Gln Val Pro Val Trp Asn  
 980 985 990  
 Asp Leu Trp Gln Gly Leu Leu Ala Gly Glu Gly Leu Ser Ser Glu Leu  
 995 1000 1005  
 20 Gln Lys Leu Asp Ala Ile Trp Leu Ala Arg Gly Gly Ile Gly Leu Glu  
 1010 1015 1020  
 Ala Ile Arg Thr Val Ser Leu Asp Thr Leu Phe Gly Thr Gly Thr Leu  
 1025 1030 1035 1040  
 25 Ser Glu Asn Ile Asn Lys Val Leu Asn Gly Glu Thr Val Ser Pro Ser  
 1045 1050 1055  
 Gly Gly Val Thr Leu Ala Leu Thr Gly Asp Ile Phe Gln Ala Thr Leu  
 1060 1065 1070  
 30 Asp Leu Ser Gln Leu Gly Leu Asp Asn Ser Tyr Asn Leu Gly Asn Glu  
 1075 1080 1085  
 Lys Lys Arg Arg Ile Lys Arg Ile Ala Val Thr Leu Pro Thr Leu Leu  
 1090 1095 1100  
 Gly Pro Tyr Gln Asp Leu Glu Ala Thr Leu Val Met Gly Ala Glu Ile  
 1105 1110 1115 1120  
 40 Ala Ala Leu Ser His Gly Val Asn Asp Gly Gly Arg Phe Val Thr Asp  
 1125 1130 1135  
 Phe Asn Asp Ser Arg Phe Leu Pro Phe Glu Gly Arg Asp Ala Thr Thr  
 1140 1145 1150  
 Gly Thr Leu Glu Leu Asn Ile Phe His Ala Gly Lys Glu Gly Thr Gln  
 1155 1160 1165  
 50 His Glu Leu Val Ala Asn Leu Ser Asp Ile Ile Val His Leu Asn Tyr  
 1170 1175 1180  
 Ile Ile Arg Asp Ala \*  
 1185 1190  
 55

## (2) INFORMATION FOR SEQ ID NO:27:

- 60 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1881 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear  
 65 (ii) MOLECULE TYPE: DNA (genomic)

## (ix) FEATURE:

5

(A) NAME/KEY: CDS

(B) LOCATION: 1..1881

(D) OTHER INFORMATION: product = "P8"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

10 ATG TCT GAA TCT TTA TTT ACA CAA ACG TTG AAA GAA GCG CGC CGT GAT 48  
Met Ser Glu Ser Leu Phe Thr Gln Thr Leu Lys Glu Ala Arg Arg Asp  
1 5 10 15

15 GCA TTG GTT GCT CAT TAT ATT GCT ACT CAG GTG CCC GCA GAT TTA AAA 96  
Ala Leu Val Ala His Tyr Ile Ala Thr Gln Val Pro Ala Asp Leu Lys  
20 25 30

20 GAG AGT ATC CAG ACC GCG GAT GAT CTG TAC GAA TAT CTG TTG CTG GAT 144  
Glu Ser Ile Gln Thr Ala Asp Asp Leu Tyr Glu Tyr Leu Leu Leu Asp  
35 40 45

25 ACC AAA ATT AGC GAT CTG GTT ACT ACT TCA CCG CTG TCC GAA GCG ATT 192  
Thr Lys Ile Ser Asp Leu Val Thr Thr Ser Pro Leu Ser Glu Ala Ile  
50 55 60

30 GGC AGT CTG CAA TTG TTT ATT CAT CGT GCG ATA GAG GGC TAT GAC GGC 240  
Gly Ser Leu Gln Leu Phe Ile His Arg Ala Ile Glu Gly Tyr Asp Gly  
65 70 75 80

35 ACG CTG GCA GAC TCA GCA AAA CCC TAT TTT GCC GAT GAA CAG TTT TTA 288  
Thr Leu Ala Asp Ser Ala Lys Pro Tyr Phe Ala Asp Glu Gln Phe Leu  
85 90 95

40 TAT AAC TGG GAT AGT TTT AAC CAC CGT TAT AGC ACT TGG GCT GGC AAG 336  
Tyr Asn Trp Asp Ser Phe Asn His Arg Tyr Ser Thr Trp Ala Gly Lys  
100 105 110

45 GAA CGG TTG AAA TTC TAT GCC GGG GAT TAT ATT GAT CCA ACA TTG CGA 384  
Glu Arg Leu Lys Phe Tyr Ala Gly Asp Tyr Ile Asp Pro Thr Leu Arg  
115 120 125

50 TTG AAT AAG ACC GAG ATA TTT ACC GCA TTT GAA CAA GGT ATT TCT CAA 432  
Leu Asn Lys Thr Glu Ile Phe Thr Ala Phe Glu Gln Gly Ile Ser Gln  
130 135 140

55 GGG AAA TTA AAA AGT GAA TTA GTC GAA TCT AAA TTA CGT GAT TAT CTA 480  
Gly Lys Leu Lys Ser Glu Leu Val Glu Ser Lys Leu Arg Asp Tyr Leu  
145 150 155 160

60 ATT AGT TAT GAC ACT TTA GCC ACC CTT GAT TAT ATT ACT GCC TGC CAA 528  
Ile Ser Tyr Asp Thr Leu Ala Thr Leu Asp Tyr Ile Thr Ala Cys Gln  
165 170 175

65 GGC AAA GAT AAT AAA ACC ATC TTC TTT ATT GGC CGT ACA CAG AAT GCA 576  
Gly Lys Asp Asn Lys Thr Ile Phe Phe Ile Gly Arg Thr Gln Asn Ala  
180 185 190

60 CCC TAT GCA TTT TAT TGG CGA AAA TTA ACT TTA GTC ACT GAT GGC GGT 624  
Pro Tyr Ala Phe Tyr Trp Arg Lys Leu Thr Leu Val Thr Asp Gly Gly  
195 200 205

65 AAG TTG AAA CCA GAT CAA TGG TCA GAG TGG CGA GCA ATT AAT GCC GGG 672  
Lys Leu Lys Pro Asp Gln Trp Ser Glu Trp Arg Ala Ile Asn Ala Gly  
210 215 220

5	ATT	AST	GAG	GCA	TAT	TCA	GGG	CAT	GTC	GAG	CCT	TTC	TGG	GAA	AAT	AAC	711
	Ile	Ser	Glu	Ala	Tyr	Ser	Gly	His	Val	Glu	Pro	Phe	Trp	Glu	Asn	Asn	225
10	AAG	CTG	CAC	ATC	CGT	TGG	TTT	ACT	ATC	TCG	AAA	GAA	GAT	AAA	ATA	GAT	758
	Lys	Leu	His	Ile	Arg	Trp	Phe	Thr	Ile	Ser	Lys	Glu	Asp	Lys	Ile	Asp	235
15	TTT	GTT	TAT	AAA	AAC	ATC	TGG	GTG	ATG	AGT	AGC	GAT	TAT	AGC	TGG	GCA	816
	Phe	Val	Tyr	Lys	Asn	Ile	Trp	Val	Met	Ser	Ser	Asp	Tyr	Ser	Trp	Ala	240
20	TCA	AAG	AAA	AAA	ATC	TTG	GAA	CTT	TCT	TTT	ACT	GAC	TAC	AAT	AGA	GTT	864
	Ser	Lys	Lys	Lys	Ile	Leu	Glu	Leu	Ser	Phe	Thr	Asp	Tyr	Asn	Arg	Val	255
25	GGA	GCA	ACA	GGA	TCA	TCA	AGC	CCG	ACT	GAA	GTA	GCT	TCA	CAA	TAT	GGT	912
	Gly	Ala	Thr	Gly	Ser	Ser	Ser	Pro	Thr	Glu	Val	Ala	Ser	Gln	Tyr	Gly	260
30	TCT	GAT	GCT	CAG	ATG	AAT	ATT	TCT	GAT	GAT	GGG	ACT	GTA	CTT	ATT	TTT	960
	Ser	Asp	Ala	Gln	Met	Asn	Ile	Ser	Asp	Asp	Gly	Thr	Val	Leu	Ile	Phe	265
35	CAG	AAT	GCC	GGC	GGA	GCT	ACT	CCC	AGT	ACT	GGA	GTG	ACG	TTA	TGT	TAT	1008
	Gln	Asn	Ala	Gly	Gly	Ala	Thr	Pro	Ser	Thr	Gly	Val	Thr	Leu	Cys	Tyr	310
40	GAC	TCT	GGC	AAC	GTG	ATT	AAG	AAC	CTA	TCT	AGT	ACA	GGA	AGT	GCA	AAT	1056
	Asp	Ser	Gly	Asn	Val	Ile	Lys	Asn	Leu	Ser	Ser	Thr	Gly	Ser	Ala	Asn	315
45	TTA	TCG	TCA	AAG	GAT	TAT	GCC	ACA	ACT	AAA	TTA	CGC	ATG	TGT	CAT	GGA	1104
	Leu	Ser	Ser	Lys	Asp	Tyr	Ala	Thr	Thr	Lys	Leu	Arg	Met	Cys	His	Gly	320
50	CAA	AGT	TAC	AAT	GAT	AAT	AAC	TAC	TGC	AAT	TTT	ACA	CTC	TCT	ATT	AAT	1152
	Gln	Ser	Tyr	Asn	Asp	Asn	Asn	Tyr	Cys	Asn	Phe	Thr	Leu	Ser	Ile	Asn	325
55	ACA	ATA	GAA	TTC	ACC	TCC	TAC	GGC	ACA	TTC	TCA	TCA	GAT	GGA	AAA	CAA	1200
	Thr	Ile	Glu	Phe	Thr	Ser	Tyr	Gly	Thr	Phe	Ser	Ser	Asp	Gly	Lys	Gln	330
60	TTT	ACA	CCA	CCT	TCT	GGT	TCT	GCC	ATT	GAT	TTA	CAC	CTC	CCT	AAT	TAT	1248
	Phe	Thr	Pro	Pro	Ser	Gly	Ser	Ala	Ile	Asp	Leu	His	Leu	Pro	Asn	Tyr	335
65	GTA	GAT	CTC	AAC	GCG	CTA	TTA	GAT	ATT	AGC	CTC	GAT	TCA	CTA	CTT	AAT	1296
	Val	Asp	Leu	Asn	Ala	Leu	Leu	Asp	Ile	Ser	Leu	Asp	Ser	Leu	Leu	Asn	340
70	TAT	GAC	GTT	CAG	GGG	CAG	TTT	GGC	GGA	TCT	AAT	CCG	GTT	GAT	AAT	TTC	1344
	Tyr	Asp	Val	Gln	Gly	Gln	Phe	Gly	Gly	Ser	Asn	Pro	Val	Asp	Asn	Phe	345
75	AGT	GGT	CCC	TAT	GGT	ATT	TAT	CTA	TGG	GAA	ATC	TTC	TTC	CAT	ATT	CCG	1392
	Ser	Gly	Pro	Tyr	Gly	Ile	Tyr	Leu	Trp	Glu	Ile	Phe	Phe	His	Ile	Pro	350
80	TTC	CTT	GTT	ACG	GTC	CGT	ATG	CAA	ACC	GAA	CAA	CGT	TAC	GAA	GAC	GCG	1440
	Phe	Leu	Val	Thr	Val	Arg	Met	Gln	Thr	Glu	Gln	Arg	Tyr	Glu	Asp	Ala	355
85	GAC	ACT	TGG	TAC	AAA	TAT	ATT	TTC	CGC	AGC	GCC	GGT	TAT	CGC	GAT	GCT	1488

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      Asp Thr Trp Tyr Lys Tyr Ile Phe Arg Ser Ala Gly Tyr Arg Asp Ala
      485                                490                                495

5    AAT GGC CAG CTC ATT ATG GAT GGC AGT AAA CCA CGT TAT TGG AAT GTG 1536
     Asn Gly Gln Leu Ile Met Asp Gly Ser Lys Pro Arg Tyr Trp Asn Val
      500                                505                                510

10   ATG CCA TTG CAA CTG GAT ACC GCA TGG GAT ACC ACA CAG CCC GCC ACC 1584
     Met Pro Leu Gln Leu Asp Thr Ala Trp Asp Thr Thr Gln Pro Ala Thr
      515                                520                                525

15   ACT GAT CCA GAT GTG ATC GCT ATG GCG GAC CCG ATG CAT TAC AAG CTG 1632
     Thr Asp Pro Asp Val Ile Ala Met Ala Asp Pro Met His Tyr Lys Leu
      530                                535                                540

20   GCG ATA TTC CTG CAT ACC CTT GAT CTA TTG ATT GCC CGA GGC GAC AGC 1680
     Ala Ile Phe Leu His Thr Leu Asp Leu Leu Ile Ala Arg Gly Asp Ser
      545                                550                                555

25   GCT TAC CGT CAA CTT GAA CGC GAT ACT CTA GTC GAA GCC AAA ATG TAC 1728
     Ala Tyr Arg Gln Leu Glu Arg Asp Thr Leu Val Glu Ala Lys Met Tyr
      565                                570                                575

30   TAC ATT CAG GCA CAA CAG CTA CTG GGA CCG CGC CCT GAT ATC CAT ACC 1776
     Tyr Ile Gln Ala Gln Gln Leu Leu Gly Pro Arg Pro Asp Ile His Thr
      580                                585                                590

35   ACC AAT ACT TGG CCA AAT CCC ACC TTG AGT AAA GAA GCT GGC GCT ATT 1824
     Thr Asn Thr Trp Pro Asn Pro Thr Leu Ser Lys Glu Ala Gly Ala Ile
      595                                600                                605

40   GCC ACA CCG ACA TTC CTC AGT TCA CCG GAG GTG ATG ACG TTC GCT GCC 1872
     Ala Thr Pro Thr Phe Leu Ser Ser Pro Glu Val Met Thr Phe Ala Ala
      610                                615                                620

45   TGG CTA AGC
     Trp Leu Ser
      625

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1881

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(2) INFORMATION FOR SEQ ID NO:28:

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      (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 627 amino acids
          (B) TYPE: amino acid
          (D) TOPOLOGY: linear

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45

(ii) MOLECULE TYPE: protein

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

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Met Ser Glu Ser Leu Phe Thr Gln Thr Leu Lys Glu Ala Arg Arg Asp
 1          5          10          15

55   Ala Leu Val Ala His Tyr Ile Ala Thr Gln Val Pro Ala Asp Leu Lys
      20          25          30

60   Glu Ser Ile Gln Thr Ala Asp Asp Leu Tyr Glu Tyr Leu Leu Asp
      35          40          45

      Thr Lys Ile Ser Asp Leu Val Thr Thr Ser Pro Leu Ser Glu Ala Ile
      50          55          60

65   Gly Ser Leu Gln Leu Phe Ile His Arg Ala Ile Glu Gly Tyr Asp Gly

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	65		70		75		80									
	Thr	Leu	Ala	Asp	Ser	Ala	Lys	Pro	Tyr	Phe	Ala	Asp	Glu	Gln	Phe	Leu
					85					90					95	
5	Tyr	Asn	Trp	Asp	Ser	Phe	Asn	His	Arg	Tyr	Ser	Thr	Trp	Ala	Gly	Lys
				100					105					110		
10	Glu	Arg	Leu	Lys	Phe	Tyr	Ala	Gly	Asp	Tyr	Ile	Asp	Pro	Thr	Leu	Arg
			115					120				125				
	Leu	Asn	Lys	Thr	Glu	Ile	Phe	Thr	Ala	Phe	Glu	Gln	Gly	Ile	Ser	Gln
	130						135					140				
15	Gly	Lys	Leu	Lys	Ser	Glu	Leu	Val	Glu	Ser	Lys	Leu	Arg	Asp	Tyr	Leu
	145					150					155					160
	Ile	Ser	Tyr	Asp	Thr	Leu	Ala	Thr	Leu	Asp	Tyr	Ile	Thr	Ala	Cys	Gln
					165					170					175	
20	Gly	Lys	Asp	Asn	Lys	Thr	Ile	Phe	Phe	Ile	Gly	Arg	Thr	Gln	Asn	Ala
				180					185					190		
	Pro	Tyr	Ala	Phe	Tyr	Trp	Arg	Lys	Leu	Thr	Leu	Val	Thr	Asp	Gly	Gly
25			195					200					205			
	Lys	Leu	Lys	Pro	Asp	Gln	Trp	Ser	Glu	Trp	Arg	Ala	Ile	Asn	Ala	Gly
	210						215					220				
30	Ile	Ser	Glu	Ala	Tyr	Ser	Gly	His	Val	Glu	Pro	Phe	Trp	Glu	Asn	Asn
	225					230				235						240
	Lys	Leu	His	Ile	Arg	Trp	Phe	Thr	Ile	Ser	Lys	Glu	Asp	Lys	Ile	Asp
				245					250						255	
35	Phe	Val	Tyr	Lys	Asn	Ile	Trp	Val	Met	Ser	Ser	Asp	Tyr	Ser	Trp	Ala
				260					265					270		
	Ser	Lys	Lys	Lys	Ile	Leu	Glu	Leu	Ser	Phe	Thr	Asp	Tyr	Asn	Arg	Val
40			275					280					285			
	Gly	Ala	Thr	Gly	Ser	Ser	Ser	Pro	Thr	Glu	Val	Ala	Ser	Gln	Tyr	Gly
	290						295					300				
45	Ser	Asp	Ala	Gln	Met	Asn	Ile	Ser	Asp	Asp	Gly	Thr	Val	Leu	Ile	Phe
	305					310					315					320
	Gln	Asn	Ala	Gly	Gly	Ala	Thr	Pro	Ser	Thr	Gly	Val	Thr	Leu	Cys	Tyr
				325						330					335	
50	Asp	Ser	Gly	Asn	Val	Ile	Lys	Asn	Leu	Ser	Ser	Thr	Gly	Ser	Ala	Asn
				340					345					350		
	Leu	Ser	Ser	Lys	Asp	Tyr	Ala	Thr	Thr	Lys	Leu	Arg	Met	Cys	His	Gly
55			355					360					365			
	Gln	Ser	Tyr	Asn	Asp	Asn	Asn	Tyr	Cys	Asn	Phe	Thr	Leu	Ser	Ile	Asn
	370						375					380				
60	Thr	Ile	Glu	Phe	Thr	Ser	Tyr	Gly	Thr	Phe	Ser	Ser	Asp	Gly	Lys	Gln
	385					390					395					400
	Phe	Thr	Pro	Pro	Ser	Gly	Ser	Ala	Ile	Asp	Leu	His	Leu	Pro	Asn	Tyr
				405						410					415	
65	Val	Asp	Leu	Asn	Ala	Leu	Leu	Asp	Ile	Ser	Leu	Asp	Ser	Leu	Leu	Asn



420                      425                      430  
 Tyr Asp Val Gln Gly Gln Phe Gly Gly Ser Asn Pro Val Asp Asn Phe  
                          435                      440                      445  
 5 Ser Gly Pro Tyr Gly Ile Tyr Leu Trp Glu Ile Phe Phe His Ile Pro  
                          450                      455                      460  
 10 Phe Leu Val Thr Val Arg Met Gln Thr Glu Gln Arg Tyr Glu Asp Ala  
                          465                      470                      475                      480  
 Asp Thr Trp Tyr Lys Tyr Ile Phe Arg Ser Ala Gly Tyr Arg Asp Ala  
    485                      490                      495  
 15 Asn Gly Gln Leu Ile Met Asp Gly Ser Lys Pro Arg Tyr Trp Asn Val  
    500                      505                      510  
 20 Met Pro Leu Gln Leu Asp Thr Ala Trp Asp Thr Thr Gln Pro Ala Thr  
    515                      520                      525  
 Thr Asp Pro Asp Val Ile Ala Met Ala Asp Pro Met His Tyr Lys Leu  
    530                      535                      540  
 25 Ala Ile Phe Leu His Thr Leu Asp Leu Leu Ile Ala Arg Gly Asp Ser  
    545                      550                      555                      560  
 Ala Tyr Arg Gln Leu Glu Arg Asp Thr Leu Val Glu Ala Lys Met Tyr  
    565                      570                      575  
 30 Tyr Ile Gln Ala Gln Gln Leu Leu Gly Pro Arg Pro Asp Ile His Thr  
    580                      585                      590  
 Thr Asn Thr Trp Pro Asn Pro Thr Leu Ser Lys Glu Ala Gly Ala Ile  
    595                      600                      605  
 35 Ala Thr Pro Thr Phe Leu Ser Ser Pro Glu Val Met Thr Phe Ala Ala  
    610                      615                      620  
 40 Trp Leu Ser  
    625

## (2) INFORMATION FOR SEQ ID NO:29:

- 45 (i) SEQUENCE CHARACTERISTICS:  
     (A) LENGTH: 1689 base pairs  
     (B) TYPE: nucleic acid  
     (C) STRANDEDNESS: double  
     (D) TOPOLOGY: linear  
 50 (ii) MOLECULE TYPE: DNA (genomic)  
 55 (ix) FEATURE:  
     (A) NAME/KEY: CDS  
     (B) LOCATION: 1..1689  
     (D) OTHER INFORMATION: /product= "S8"  
 60 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GCA GGC GAT ACC GCA AAT ATT GGC GAC GGT GAT TTC TTG CCA CCG TAC    48  
 Ala Gly Asp Thr Ala Asn Ile Gly Asp Gly Asp Phe Leu Pro Pro Tyr  
     1                      5                      10                      15

	AAC	GAT	CTA	CTA	CTC	GGT	TAC	TGG	GAT	AAA	CTT	GAG	TTA	CGC	CTA	TAC	36
	Asn	Asp	Val	Leu	Leu	Gly	Tyr	Trp	Asp	Lys	Leu	Glu	Leu	Arg	Leu	Tyr	
			20						25					30			
5	AAC	CTG	CGC	CAC	AAT	CTG	AGT	CTG	GAT	GGT	CAA	CCG	CTA	AAT	CTG	CCA	144
	Asn	Leu	Arg	His	Asn	Leu	Ser	Leu	Asp	Gly	Gln	Pro	Leu	Asn	Leu	Pro	
			35					40					45				
10	CTG	TAT	GCC	ACG	CCG	GTA	GAC	CCG	AAA	ACC	CTG	CAA	CGC	CAG	GAA	GCC	192
	Leu	Tyr	Ala	Thr	Pro	Val	Asp	Pro	Lys	Thr	Leu	Gln	Arg	Gln	Gln	Ala	
		50					55					60					
15	GGA	GGG	GAC	GGT	ACA	GGC	AGT	AGT	CCG	GCT	GGT	GGT	CAA	GGC	AGT	GTT	240
	Gly	Gly	Asp	Gly	Thr	Gly	Ser	Ser	Pro	Ala	Gly	Gly	Gln	Gly	Ser	Val	
	65					70					75					80	
20	CAG	GGC	TGG	CGC	TAT	CCG	TTA	TTG	GTA	GAA	CGC	GCC	CGC	TCT	GCC	GTG	288
	Gln	Gly	Trp	Arg	Tyr	Pro	Leu	Leu	Val	Glu	Arg	Ala	Arg	Ser	Ala	Val	
					85					90					95		
25	AGT	TTG	TTG	ACT	CAG	TTC	GGC	AAC	AGC	TTA	CAA	ACA	ACG	TTA	GAA	CAT	336
	Ser	Leu	Leu	Thr	Gln	Phe	Gly	Asn	Ser	Leu	Gln	Thr	Thr	Leu	Glu	His	
				100					105					110			
30	CAG	GAT	AAT	GAA	AAA	ATG	ACG	ATA	CTG	TTG	CAG	ACT	CAA	CAG	GAA	GCC	384
	Gln	Asp	Asn	Glu	Lys	Met	Thr	Ile	Leu	Leu	Gln	Thr	Gln	Gln	Glu	Ala	
			115					120					125				
35	ATC	CTG	AAA	CAT	CAG	CAC	GAT	ATA	CAA	CAA	AAT	AAT	CTA	AAA	GGA	TTA	432
	Ile	Leu	Lys	His	Gln	His	Asp	Ile	Gln	Gln	Asn	Asn	Leu	Lys	Gly	Leu	
		130					135					140					
40	CAA	CAC	AGC	CTG	ACC	GCA	TTA	CAG	GCT	AGC	CGT	GAT	GGC	GAC	ACA	TTG	480
	Gln	His	Ser	Leu	Thr	Ala	Leu	Gln	Ala	Ser	Arg	Asp	Gly	Asp	Thr	Leu	
	145					150					155					160	
45	CGG	CAA	AAA	CAT	TAC	AGC	GAC	CTG	ATT	AAC	GGT	GGT	CTA	TCT	GCG	GCA	528
	Arg	Gln	Lys	His	Tyr	Ser	Asp	Leu	Ile	Asn	Gly	Gly	Leu	Ser	Ala	Ala	
				165					170						175		
50	GAA	ATC	GCC	GGT	CTG	ACA	CTA	CGC	AGC	ACC	GCC	ATG	ATT	ACC	AAT	GGC	576
	Glu	Ile	Ala	Gly	Leu	Thr	Leu	Arg	Ser	Thr	Ala	Met	Ile	Thr	Asn	Gly	
			180						185					190			
55	GTT	GCA	ACG	GGA	TTG	CTG	ATT	GCC	GGC	GGA	ATC	GCC	AAC	GCG	GTA	CCT	624
	Val	Ala	Thr	Gly	Leu	Leu	Ile	Ala	Gly	Gly	Ile	Ala	Asn	Ala	Val	Pro	
			195					200					205				
60	AAC	GTC	TTC	GGG	CTG	GCT	AAC	GGT	GGA	TCG	GAA	TGG	GGA	GCG	CCA	TTA	672
	Asn	Val	Phe	Gly	Leu	Ala	Asn	Gly	Gly	Ser	Glu	Trp	Gly	Ala	Pro	Leu	
		210					215					220					
65	ATT	GGC	TCC	GGG	CAA	GCA	ACC	CAA	GTT	GGC	GCC	GGC	ATC	CAG	GAT	CAG	720
	Ile	Gly	Ser	Gly	Gln	Ala	Thr	Gln	Val	Gly	Ala	Gly	Ile	Gln	Asp	Gln	
	225					230					235					240	
70	AGC	GCG	GGC	ATT	TCA	GAA	GTG	ACA	GCA	GGC	TAT	CAG	CGT	CGT	CAG	GAA	768
	Ser	Ala	Gly	Ile	Ser	Glu	Val	Thr	Ala	Gly	Tyr	Gln	Arg	Arg	Gln	Glu	
				245						250					255		
75	GAA	TGG	GCA	TTG	CAA	CGG	GAT	ATT	GCT	GAT	AAC	GAA	ATA	ACC	CAA	CTG	816
	Glu	Trp	Ala	Leu	Gln	Arg	Asp	Ile	Ala	Asp	Asn	Glu	Ile	Thr	Gln	Leu	
			260						265					270			
80	GAT	GCC	CAG	ATA	CAA	AGC	CTG	CAA	GAG	CAA	ATC	ACG	ATG	GCA	CAA	AAA	864

	Asp	Ala	Gln	Ile	Gln	Ser	Leu	Gln	Glu	Gln	Ile	Thr	Met	Ala	Gln	Lys	
			275					280					285				
5	CAG	ATC	ACG	CTC	TCT	GAA	ACC	GAA	CAA	GCG	AAT	GCC	CAA	GCG	ATT	TAT	912
	Gln	Ile	Thr	Leu	Ser	Glu	Thr	Glu	Gln	Ala	Asn	Ala	Gln	Ala	Ile	Tyr	
			290				295					300					
10	GAC	CTG	CAA	ACC	ACT	CGT	TTT	ACC	GGG	CAG	GCA	CTG	TAT	AAC	TGG	ATG	960
	Asp	Leu	Gln	Thr	Thr	Arg	Phe	Thr	Gly	Gln	Ala	Leu	Tyr	Asn	Trp	Met	
			305			310					315					320	
15	GCC	GGT	CGT	CTC	TCC	GCG	CTC	TAT	TAC	CAA	ATG	TAT	GAT	TCC	ACT	CTG	1008
	Ala	Gly	Arg	Leu	Ser	Ala	Leu	Tyr	Tyr	Gln	Met	Tyr	Asp	Ser	Thr	Leu	
					325					330					335		
20	CCA	ATC	TGT	CTC	CAG	CCA	AAA	GCC	GCA	TTA	GTA	CAG	GAA	TTA	GGC	GAG	1056
	Pro	Ile	Cys	Leu	Gln	Pro	Lys	Ala	Ala	Leu	Val	Gln	Glu	Leu	Gly	Glu	
				340					345					350			
25	AAA	GAG	AGC	GAC	AGT	CTT	TTC	CAG	GTT	CCG	GTG	TGG	AAT	GAT	CTG	TGG	1104
	Lys	Glu	Ser	Asp	Ser	Leu	Phe	Gln	Val	Pro	Val	Trp	Asn	Asp	Leu	Trp	
			355				360						365				
30	CAA	GGG	CTG	TTA	GCA	GGA	GAA	GGT	TTA	AGT	TCA	GAG	CTA	CAG	AAA	CTG	1152
	Gln	Gly	Leu	Leu	Ala	Gly	Glu	Gly	Leu	Ser	Ser	Glu	Leu	Gln	Lys	Leu	
			370				375					380					
35	GAT	GCC	ATC	TGG	CTT	GCA	CGT	GGT	GGT	ATT	GGG	CTA	GAA	GCC	ATC	CGC	1200
	Asp	Ala	Ile	Trp	Leu	Ala	Arg	Gly	Gly	Ile	Gly	Leu	Glu	Ala	Ile	Arg	
			385			390					395					400	
40	ACC	GTG	TCG	CTG	GAT	ACC	CTG	TTT	GGC	ACA	GGG	ACG	TTA	AGT	GAA	AAT	1248
	Thr	Val	Ser	Leu	Asp	Thr	Leu	Phe	Gly	Thr	Gly	Thr	Leu	Ser	Glu	Asn	
				405					410						415		
45	ATC	AAT	AAA	GTG	CTT	AAC	GGG	GAA	ACG	GTA	TCT	CCA	TCC	GGT	GGC	GTC	1296
	Ile	Asn	Lys	Val	Leu	Asn	Gly	Glu	Thr	Val	Ser	Pro	Ser	Gly	Gly	Val	
				420					425					430			
50	ACT	CTG	GCG	CTG	ACA	GGG	GAT	ATC	TTC	CAA	GCA	ACA	CTG	GAT	TTG	AGT	1344
	Thr	Leu	Ala	Leu	Thr	Gly	Asp	Ile	Phe	Gln	Ala	Thr	Leu	Asp	Leu	Ser	
			435				440						445				
55	CAG	CTA	GGT	TTG	GAT	AAC	TCT	TAC	AAC	TTG	GGT	AAC	GAG	AAG	AAA	CGT	1392
	Gln	Leu	Gly	Leu	Asp	Asn	Ser	Tyr	Asn	Leu	Gly	Asn	Glu	Lys	Lys	Arg	
		450				455					460						
60	CGT	ATT	AAA	CGT	ATC	GCC	GTC	ACC	CTG	CCA	ACA	CTT	CTG	GGG	CCA	TAT	1440
	Arg	Ile	Lys	Arg	Ile	Ala	Val	Thr	Leu	Pro	Thr	Leu	Leu	Gly	Pro	Tyr	
		465				470				475					480		
65	CAA	GAT	CTT	GAA	GCC	ACA	CTG	GTA	ATG	GGT	GCG	GAA	ATC	GCC	GCC	TTA	1488
	Gln	Asp	Leu	Glu	Ala	Thr	Leu	Val	Met	Gly	Ala	Glu	Ile	Ala	Ala	Leu	
				485					490					495			
70	TCA	CAC	GGT	GTG	AAT	GAC	GGA	GGC	CGG	TTT	GTT	ACC	GAC	TTT	AAC	GAC	1536
	Ser	His	Gly	Val	Asn	Asp	Gly	Gly	Arg	Phe	Val	Thr	Asp	Phe	Asn	Asp	
				500					505					510			
75	AGC	CGT	TTT	CTG	CCT	TTT	GAA	GGT	CGA	GAT	GCA	ACA	ACC	GGC	ACA	CTG	1584
	Ser	Arg	Phe	Leu	Pro	Phe	Glu	Gly	Arg	Asp	Ala	Thr	Thr	Gly	Thr	Leu	
			515					520					525				
80	GAG	CTC	AAT	ATT	TTC	CAT	GCG	GGT	AAA	GAG	GGA	ACG	CAA	CAC	GAG	TTG	1632
	Glu	Leu	Asn	Ile	Phe	His	Ala	Gly	Lys	Glu	Gly	Thr	Gln	His	Glu	Leu	
		530					535					540					

CTC GCG AAT CTG AGT GAC ATC ATT GTG CAT CTG AAT TAC ATC ATT CGA 1680  
 Val Ala Asn Leu Ser Asp Ile Ile Val His Leu Asn Tyr Ile Ile Arg  
 545 550 555 560

5

GAC GCG TAA  
 Asp Ala \*

1689

10

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

15

(A) LENGTH: 563 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Ala Gly Asp Thr Ala Asn Ile Gly Asp Gly Asp Phe Leu Pro Pro Tyr  
 1 5 10 15  
 Asn Asp Val Leu Leu Gly Tyr Trp Asp Lys Leu Glu Leu Arg Leu Tyr  
 20 25 30  
 Asn Leu Arg His Asn Leu Ser Leu Asp Gly Gln Pro Leu Asn Leu Pro  
 35 40 45  
 Leu Tyr Ala Thr Pro Val Asp Pro Lys Thr Leu Gln Arg Gln Gln Ala  
 50 55 60  
 Gly Gly Asp Gly Thr Gly Ser Ser Pro Ala Gly Gly Gln Gly Ser Val  
 65 70 75 80  
 Gln Gly Trp Arg Tyr Pro Leu Leu Val Glu Arg Ala Arg Ser Ala Val  
 85 90 95  
 Ser Leu Leu Thr Gln Phe Gly Asn Ser Leu Gln Thr Thr Leu Glu His  
 100 105 110  
 Gln Asp Asn Glu Lys Met Thr Ile Leu Leu Gln Thr Gln Gln Glu Ala  
 115 120 125  
 Ile Leu Lys His Gln His Asp Ile Gln Gln Asn Asn Leu Lys Gly Leu  
 130 135 140  
 Gln His Ser Leu Thr Ala Leu Gln Ala Ser Arg Asp Gly Asp Thr Leu  
 145 150 155 160  
 Arg Gln Lys His Tyr Ser Asp Leu Ile Asn Gly Gly Leu Ser Ala Ala  
 165 170 175  
 Glu Ile Ala Gly Leu Thr Leu Arg Ser Thr Ala Met Ile Thr Asn Gly  
 180 185 190  
 Val Ala Thr Gly Leu Leu Ile Ala Gly Gly Ile Ala Asn Ala Val Pro  
 195 200 205  
 Asn Val Phe Gly Leu Ala Asn Gly Gly Ser Glu Trp Gly Ala Pro Leu  
 210 215 220  
 Ile Gly Ser Gly Gln Ala Thr Gln Val Gly Ala Gly Ile Gln Asp Gln

	225		230		235		240									
	Ser	Ala	Gly	Ile	Ser	Glu	Val	Thr	Ala	Gly	Tyr	Gln	Arg	Arg	Gln	Glu
					245					250					255	
5	Glu	Trp	Ala	Leu	Gln	Arg	Asp	Ile	Ala	Asp	Asn	Glu	Ile	Thr	Gln	Leu
			260						265					270		
10	Asp	Ala	Gln	Ile	Gln	Ser	Leu	Gln	Glu	Gln	Ile	Thr	Met	Ala	Gln	Lys
			275					280					285			
	Gln	Ile	Thr	Leu	Ser	Glu	Thr	Glu	Gln	Ala	Asn	Ala	Gln	Ala	Ile	Tyr
			290				295					300				
15	Asp	Leu	Gln	Thr	Thr	Arg	Phe	Thr	Gly	Gln	Ala	Leu	Tyr	Asn	Trp	Met
	305					310					315					320
	Ala	Gly	Arg	Leu	Ser	Ala	Leu	Tyr	Tyr	Gln	Met	Tyr	Asp	Ser	Thr	Leu
					325					330					335	
20	Pro	Ile	Cys	Leu	Gln	Pro	Lys	Ala	Ala	Leu	Val	Gln	Glu	Leu	Gly	Glu
			340						345					350		
25	Lys	Glu	Ser	Asp	Ser	Leu	Phe	Gln	Val	Pro	Val	Trp	Asn	Asp	Leu	Trp
			355				360						365			
	Gln	Gly	Leu	Leu	Ala	Gly	Glu	Gly	Leu	Ser	Ser	Glu	Leu	Gln	Lys	Leu
		370					375					380				
30	Asp	Ala	Ile	Trp	Leu	Ala	Arg	Gly	Gly	Ile	Gly	Leu	Glu	Ala	Ile	Arg
	385					390					395					400
	Thr	Val	Ser	Leu	Asp	Thr	Leu	Phe	Gly	Thr	Gly	Thr	Leu	Ser	Glu	Asn
				405						410					415	
35	Ile	Asn	Lys	Val	Leu	Asn	Gly	Glu	Thr	Val	Ser	Pro	Ser	Gly	Gly	Val
			420						425					430		
	Thr	Leu	Ala	Leu	Thr	Gly	Asp	Ile	Phe	Gln	Ala	Thr	Leu	Asp	Leu	Ser
40			435					440					445			
	Gln	Leu	Gly	Leu	Asp	Asn	Ser	Tyr	Asn	Leu	Gly	Asn	Glu	Lys	Lys	Arg
		450				455						460				
45	Arg	Ile	Lys	Arg	Ile	Ala	Val	Thr	Leu	Pro	Thr	Leu	Leu	Gly	Pro	Tyr
	465					470					475					480
	Gln	Asp	Leu	Glu	Ala	Thr	Leu	Val	Met	Gly	Ala	Glu	Ile	Ala	Ala	Leu
				485						490					495	
50	Ser	His	Gly	Val	Asn	Asp	Gly	Gly	Arg	Phe	Val	Thr	Asp	Phe	Asn	Asp
			500						505					510		
55	Ser	Arg	Phe	Leu	Pro	Phe	Glu	Gly	Arg	Asp	Ala	Thr	Thr	Gly	Thr	Leu
			515					520					525			
	Glu	Leu	Asn	Ile	Phe	His	Ala	Gly	Lys	Glu	Gly	Thr	Gln	His	Glu	Leu
		530				535						540				
60	Val	Ala	Asn	Leu	Ser	Asp	Ile	Ile	Val	His	Leu	Asn	Tyr	Ile	Ile	Arg
	545					550					555					560
	Asp	Ala														
65																

## (2) INFORMATION FOR SEQ ID NO:31:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 4458 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (ix) FEATURE:

- 15 (A) NAME/KEY: CDS  
 (B) LOCATION: 1..4458

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

20	ATG CAG GAT TCA CCA GAA GTA TCG ATT ACA ACG CTG TCA CTT CCC AAA	48
	Met Gln Asp Ser Pro Glu Val Ser Ile Thr Leu Ser Leu Pro Lys	
	1 5 10 15	
25	GGT GGC GGT GCT ATC AAT GGC ATG GGA GAA GCA CTG AAT GCT GCC GGC	96
	Gly Gly Gly Ala Ile Asn Gly Met Gly Glu Ala Leu Asn Ala Ala Gly	
	20 25 30	
30	CCT GAT GGA ATG GCC TCC CTA TCT CTG CCA TTA CCC CTT TCG ACC GGC	144
	Pro Asp Gly Met Ala Ser Leu Ser Leu Pro Leu Pro Leu Ser Thr Gly	
	35 40 45	
35	AGA GGG ACG GCT CCT GGA TTA TCG CTG ATT TAC AGC AAC AGT GCA GGT	192
	Arg Gly Thr Ala Pro Gly Leu Ser Leu Ile Tyr Ser Asn Ser Ala Gly	
	50 55 60	
40	AAT GGG CCT TTC GGC ATC GGC TGG CAA TGC GGT GTT ATG TCC ATT AGC	240
	Asn Gly Pro Phe Gly Ile Gly Trp Gln Cys Gly Val Met Ser Ile Ser	
	65 70 75 80	
45	CGA CGC ACC CAA CAT GGC ATT CCA CAA TAC GGT AAT GAC GAC ACG TTC	288
	Arg Arg Thr Gln His Gly Ile Pro Gln Tyr Gly Asn Asp Asp Thr Phe	
	85 90 95	
50	CTA TCC CCA CAA GGC GAG GTC ATG AAT ATC GCC CTG AAT GAC CAA GGG	336
	Leu Ser Pro Gln Gly Glu Val Met Asn Ile Ala Leu Asn Asp Gln Gly	
	100 105 110	
55	CAA CCT GAT ATC CGT CAA GAC GTT AAA ACG CTG CAA GGC GTT ACC TTG	384
	Gln Pro Asp Ile Arg Gln Asp Val Lys Thr Leu Gln Gly Val Thr Leu	
	115 120 125	
60	CCA ATT TCC TAT ACC GTG ACC CGC TAT CAA GCC CGC CAG ATC CTG GAT	432
	Pro Ile Ser Tyr Thr Val Thr Arg Tyr Gln Ala Arg Gln Ile Leu Asp	
	130 135 140	
65	TTC AGT AAA ATC GAA TAC TGG CAA CCT GCC TCC GGT CAA GAA GGA CGC	480
	Phe Ser Lys Ile Glu Tyr Trp Gln Pro Ala Ser Gly Gln Glu Gly Arg	
	145 150 155 160	
70	GCT TTC TGG CTG ATA TCG ACA CCG GAC GGG CAT CTA CAC ATC TTA GGG	528
	Ala Phe Trp Leu Ile Ser Thr Pro Asp Gly His Leu His Ile Leu Gly	
	165 170 175	
75	AAA ACC GCG CAG GCT TGT CTG GCA AAT CCG CAA AAT GAC CAA CAA ATC	576
	Lys Thr Ala Gln Ala Cys Leu Ala Asn Pro Gln Asn Asp Gln Gln Ile	

			180					195						190			
			GCC CAG TGG TTG CTG GAA GAA ACT GTG ACG CCA GCC GGT GAA CAT GTC	624													
5			Ala Gln Trp Leu Leu Glu Glu Thr Val Thr Pro Ala Gly Glu His Val														
			195					200						205			
			AGC TAT CAA TAT CGA GCC GAA GAT GAA GCC CAT TGT GAC GAC AAT GAA	672													
			Ser Tyr Gln Tyr Arg Ala Glu Asp Glu Ala His Cys Asp Asp Asn Glu														
			210					215						220			
10			AAA ACC GCT CAT CCC AAT GTT ACC GCA CAG CGC TAT CTG GTA CAG GTG	720													
			Lys Thr Ala His Pro Asn Val Thr Ala Gln Arg Tyr Leu Val Gln Val														
			225					230						235			240
15			AAC TAC GGC AAC ATC AAA CCA CAA GCC AGC CTG TTC GTA CTG GAT AAC	768													
			Asn Tyr Gly Asn Ile Lys Pro Gln Ala Ser Leu Phe Val Leu Asp Asn														
								245						250			255
20			GCA CCT CCC GCA CCG GAA GAG TGG CTG TTT CAT CTG GTC TTT GAC CAC	816													
			Ala Pro Pro Ala Pro Glu Glu Trp Leu Phe His Leu Val Phe Asp His														
								260						265			270
25			GGT GAG CGC GAT ACC TCA CTT CAT ACC GTG CCA ACA TGG GAT GCA GGT	864													
			Gly Glu Arg Asp Thr Ser Leu His Thr Val Pro Thr Trp Asp Ala Gly														
								275						280			285
30			ACA GCG CAA TGG TCT GTA CGC CCG GAT ATC TTC TCT CGC TAT GAA TAT	912													
			Thr Ala Gln Trp Ser Val Arg Pro Asp Ile Phe Ser Arg Tyr Glu Tyr														
								290						295			300
35			GGT TTT GAA GTG CGT ACT CGC CGC TTA TGT CAA CAA GTG CTG ATG TTT	960													
			Gly Phe Glu Val Arg Thr Arg Arg Leu Cys Gln Gln Val Leu Met Phe														
								305						310			315
40			CAC CGC ACC GCG CTC ATG GCC GGA GAA GCC AGT ACC AAT GAC GCC CCG	1008													
			His Arg Thr Ala Leu Met Ala Gly Glu Ala Ser Thr Asn Asp Ala Pro														
								325						330			335
45			GAA CTG GTT GGA CGC TTA ATA CTG GAA TAT GAC AAA AAC GCC AGC GTC	1056													
			Glu Leu Val Gly Arg Leu Ile Leu Glu Tyr Asp Lys Asn Ala Ser Val														
								340						345			350
50			ACC ACG TTG ATT ACC ATC CGT CAA TTA AGC CAT GAA TCG GAC GGG AGG	1104													
			Thr Thr Leu Ile Thr Ile Arg Gln Leu Ser His Glu Ser Asp Gly Arg														
								355						360			365
55			CCA GTC ACC CAG CCA CCA CTA GAA CTA GCC TGG CAA CGG TTT GAT CTG	1152													
			Pro Val Thr Gln Pro Pro Leu Glu Leu Ala Trp Gln Arg Phe Asp Leu														
								370						375			380
60			GAG AAA ATC CCG ACA TGG CAA CGC TTT GAC GCA CTA GAT AAT TTT AAC	1200													
			Glu Lys Ile Pro Thr Trp Gln Arg Phe Asp Ala Leu Asp Asn Phe Asn														
								385						390			395
65			TCG CAG CAA CGT TAT CAA CTG GTT GAT CTG CGG GGA GAA GGG TTG CCA	1248													
			Ser Gln Gln Arg Tyr Gln Leu Val Asp Leu Arg Gly Glu Gly Leu Pro														
								405						410			415
70			GGT ATG CTG TAT CAA GAT CGA GGC GCT TGG TGG TAT AAA GCT CCG CAA	1296													
			Gly Met Leu Tyr Gln Asp Arg Gly Ala Trp Trp Tyr Lys Ala Pro Gln														
								420						425			430
75			CGT CAG GAA GAC GGA GAC AGC AAT GCC GTC ACT TAC GAC AAA ATC GCC	1344													
			Arg Gln Glu Asp Gly Asp Ser Asn Ala Val Thr Tyr Asp Lys Ile Ala														
								435						440			445

	TTA	CTG	GCT	ACC	CTA	CCC	AAT	TTG	CAG	GAT	AAT	CCC	TCA	TTG	ATG	GAT	1330
	Pro	Leu	Pro	Thr	Leu	Pro	Asn	Leu	Gln	Asp	Asn	Ala	Ser	Leu	Met	Asp	
	450						455					460					
5	ATC	AAC	GGA	GAC	GGC	CAA	CTG	GAT	TGG	GTT	GTT	ACC	CCC	TCC	GGT	ATT	1440
	Ile	Asn	Gly	Asp	Gly	Gln	Leu	Asp	Trp	Val	Val	Thr	Ala	Ser	Gly	Ile	
	465					470					475					480	
10	CGC	GGA	TAC	CAT	AGT	CAG	CAA	CCC	GAT	GGA	AAG	TGG	ACC	CAC	TTT	ACG	1438
	Arg	Gly	Tyr	His	Ser	Gln	Gln	Pro	Asp	Gly	Lys	Trp	Thr	His	Phe	Thr	
					485					490					495		
15	CCA	ATC	AAT	GCC	TTG	CCC	GTG	GAA	TAT	TTT	CAT	CCA	AGC	ATC	CAG	TTG	1536
	Pro	Ile	Asn	Ala	Leu	Pro	Val	Glu	Tyr	Phe	His	Pro	Ser	Ile	Gln	Phe	
				500					505					510			
20	GCT	GAC	CTT	ACC	GGG	GCA	GGC	TTA	TCT	GAT	TTA	GTG	TTG	ATC	GGG	CCG	1584
	Ala	Asp	Leu	Thr	Gly	Ala	Gly	Leu	Ser	Asp	Leu	Val	Leu	Ile	Gly	Pro	
			515					520					525				
25	AAA	AGC	GTG	CGT	CTA	TAT	GCC	AAC	CAG	CGA	AAC	GGC	TGG	CGT	AAA	GGA	1632
	Lys	Ser	Val	Arg	Leu	Tyr	Ala	Asn	Gln	Arg	Asn	Gly	Trp	Arg	Lys	Gly	
		530					535					540					
30	GAA	GAT	GTC	CCC	CAA	TCC	ACA	GGT	ATC	ACC	CTG	CCT	GTC	ACA	GGG	ACC	1680
	Glu	Asp	Val	Pro	Gln	Ser	Thr	Gly	Ile	Thr	Leu	Pro	Val	Thr	Gly	Thr	
		545				550					555					560	
35	GAT	GCC	CGC	AAA	CTG	GTG	GCT	TTC	AGT	GAT	ATG	CTC	GGT	TCC	GGT	CAA	1728
	Asp	Ala	Arg	Lys	Leu	Val	Ala	Phe	Ser	Asp	Met	Leu	Gly	Ser	Gly	Gln	
					565				570						575		
40	CAA	CAT	CTG	GTG	GAA	ATC	AAG	GGT	AAT	CGC	GTC	ACC	TGT	TGG	CCG	AAT	1776
	Gln	His	Leu	Val	Glu	Ile	Lys	Gly	Asn	Arg	Val	Thr	Cys	Trp	Pro	Asn	
				580				585						590			
45	CTA	GGG	CAT	GGC	CGT	TTC	GGT	CAA	CCA	CTA	ACT	CTG	TCA	GGA	TTT	AGC	1824
	Leu	Gly	His	Gly	Arg	Phe	Gly	Gln	Pro	Leu	Thr	Leu	Ser	Gly	Phe	Ser	
			595				600						605				
50	CAG	CCC	GAA	AAT	AGC	TTC	AAT	CCC	GAA	CGG	CTG	TTT	CTG	GCG	GAT	ATC	1872
	Gln	Pro	Glu	Asn	Ser	Phe	Asn	Pro	Glu	Arg	Leu	Phe	Leu	Ala	Asp	Ile	
		610					615					620					
55	GAC	GGC	TCC	GGC	ACC	ACC	GAC	CTT	ATC	TAT	CCG	CAA	TCC	GGC	TCT	TTG	1920
	Asp	Gly	Ser	Gly	Thr	Thr	Asp	Leu	Ile	Tyr	Ala	Gln	Ser	Gly	Ser	Leu	
		625				630					635					640	
60	CTC	ATT	TAT	CTC	AAC	CAA	AGT	GGT	AAT	CAG	TTT	GAT	GCC	CCG	TTG	ACA	1968
	Leu	Ile	Tyr	Leu	Asn	Gln	Ser	Gly	Asn	Gln	Phe	Asp	Ala	Pro	Leu	Thr	
				645					650						655		
65	TTA	GCG	TTG	CCA	GAA	GGC	GTA	CAA	TTT	GAC	AAC	ACT	TGC	CAA	CTT	CAA	2016
	Leu	Ala	Leu	Pro	Glu	Gly	Val	Gln	Phe	Asp	Asn	Thr	Cys	Gln	Leu	Gln	
				660					665					670			
70	GTC	GCC	GAT	ATT	CAG	GGA	TTA	GGG	ATA	GCC	AGC	TTG	ATT	CTG	ACT	GTG	2064
	Val	Ala	Asp	Ile	Gln	Gly	Leu	Gly	Ile	Ala	Ser	Leu	Ile	Leu	Thr	Val	
			675				680						685				
75	CCA	CAT	ATC	GCG	CCA	CAT	CAC	TGG	CGT	TGT	GAC	CTG	TCA	CTG	ACC	AAA	2112
	Pro	His	Ile	Ala	Pro	His	His	Trp	Arg	Cys	Asp	Leu	Ser	Leu	Thr	Lys	
			690				695					700					
80	CCC	TGG	TTG	TTG	AAT	GTA	ATG	AAC	AAT	AAC	CGG	GGC	GCA	CAT	CAC	ACG	2160
	Pro	Trp	Leu	Leu	Asn	Val	Met	Asn	Asn	Asn	Arg	Gly	Ala	His	His	Thr	



	705					710						715				720	
	CTA	CAT	TAT	CGT	AGT	TCC	GCG	CAA	TTC	TGG	TTG	GAT	GAA	AAA	TTA	CAG	2303
5	Leu	His	Tyr	Arg	Ser	Ser	Ala	Gln	Phe	Trp	Leu	Asp	Glu	Lys	Leu	Gln	
					725					730					735		
	CTC	ACC	AAA	GCA	GCG	AAA	TCT	CCG	GCT	TGT	TAT	CTG	CCG	TTT	CCA	ATG	2356
	Leu	Thr	Lys	Ala	Gly	Lys	Ser	Pro	Ala	Cys	Tyr	Leu	Pro	Phe	Pro	Met	
10				740				745						750			
	CAT	TTG	CTA	TGG	TAT	ACC	GAA	ATT	CAG	GAT	GAA	ATC	AGC	GCG	AAC	CGG	2304
	His	Leu	Leu	Trp	Tyr	Thr	Glu	Ile	Gln	Asp	Glu	Ile	Ser	Gly	Asn	Arg	
				755				760					765				
15	CTC	ACC	AGT	GAA	GTC	AAC	TAC	AGC	CAC	GCG	GTC	TGG	GAT	GGT	AAA	GAG	2352
	Leu	Thr	Ser	Glu	Val	Asn	Tyr	Ser	His	Gly	Val	Trp	Asp	Gly	Lys	Glu	
			770				775					780					
20	CGG	GAA	TTC	AGA	GGA	TTT	GCG	TGC	ATC	AAA	CAG	ACA	GAT	ACC	ACA	ACG	2400
	Arg	Glu	Phe	Arg	Gly	Phe	Gly	Cys	Ile	Lys	Gln	Thr	Asp	Thr	Thr	Thr	
	785					790					795					800	
	TTT	TCT	CAC	GCG	ACC	GCC	CCC	GAA	CAG	GCG	GCA	CCG	TCG	CTG	AGT	ATT	2448
25	Phe	Ser	His	Gly	Thr	Ala	Pro	Glu	Gln	Ala	Ala	Pro	Ser	Leu	Ser	Ile	
					805					810					815		
	AGC	TGG	TTT	GCC	ACC	GCG	ATG	GAT	GAA	GTA	GAC	AGC	CAA	TTA	GCT	ACG	2496
	Ser	Trp	Phe	Ala	Thr	Gly	Met	Asp	Glu	Val	Asp	Ser	Gln	Leu	Ala	Thr	
30				820				825						830			
	GAA	TAT	TGG	CAG	GCA	GAC	ACG	CAA	GCT	TAT	AGC	GGA	TTT	GAA	ACC	CGT	2544
	Glu	Tyr	Trp	Gln	Ala	Asp	Thr	Gln	Ala	Tyr	Ser	Gly	Phe	Glu	Thr	Arg	
			835				840						845				
35	TAT	ACC	GTC	TGG	GAT	CAC	ACC	AAC	CAG	ACA	GAC	CAA	GCA	TTT	ACC	CCC	2592
	Tyr	Thr	Val	Trp	Asp	His	Thr	Asn	Gln	Thr	Asp	Gln	Ala	Phe	Thr	Pro	
			850				855					860					
40	AAT	GAG	ACA	CAA	CGT	AAC	TGG	CTG	ACG	CGA	GCG	CTT	AAA	GCG	CAA	CTG	2640
	Asn	Glu	Thr	Gln	Arg	Asn	Trp	Leu	Thr	Arg	Ala	Leu	Lys	Gly	Gln	Leu	
	865					870					875					880	
	CTA	CGC	ACT	GAG	CTC	TAC	GGT	CTG	GAC	GGA	ACA	GAT	AAG	CAA	ACA	GTG	2688
45	Leu	Arg	Thr	Glu	Leu	Tyr	Gly	Leu	Asp	Gly	Thr	Asp	Lys	Gln	Thr	Val	
					885					890					895		
	CCT	TAT	ACC	GTC	AGT	GAA	TCG	CGC	TAT	CAG	GTA	CGC	TCT	ATT	CCC	GTA	2736
	Pro	Tyr	Thr	Val	Ser	Glu	Ser	Arg	Tyr	Gln	Val	Arg	Ser	Ile	Pro	Val	
50				900				905						910			
	AAT	AAA	GAA	ACT	GAA	TTA	TCT	GCC	TGG	GTG	ACT	GCT	ATT	GAA	AAT	CGC	2784
	Asn	Lys	Glu	Thr	Glu	Leu	Ser	Ala	Trp	Val	Thr	Ala	Ile	Glu	Asn	Arg	
			915				920						925				
55	AGC	TAC	CAC	TAT	GAA	CGT	ATC	ATC	ACT	GAC	CCA	CAG	TTC	AGC	CAG	AGT	2832
	Ser	Tyr	His	Tyr	Glu	Arg	Ile	Ile	Thr	Asp	Pro	Gln	Phe	Ser	Gln	Ser	
			930				935					940					
60	ATC	AAG	TTG	CAA	CAC	GAT	ATC	TTT	GGT	CAA	TCA	CTG	CAA	AGT	GTC	GAT	2880
	Ile	Lys	Leu	Gln	His	Asp	Ile	Phe	Gly	Gln	Ser	Leu	Gln	Ser	Val	Asp	
	945					950					955					960	
	ATT	GCC	TGG	CCG	CGC	CGC	GAA	AAA	CCA	GCA	GTG	AAT	CCC	TAC	CCG	CCT	2928
65	Ile	Ala	Trp	Pro	Arg	Arg	Glu	Lys	Pro	Ala	Val	Asn	Pro	Tyr	Pro	Pro	
					965					970					975		

	ACC	CTG	CCG	GAA	ACC	CTA	TTT	GAG	AGC	AGC	TAT	GAT	GAT	CAA	CAA	CAA	3976
	Thr	Leu	Pro	Glu	Thr	Leu	Phe	Asp	Ser	Ser	Tyr	Asp	Asp	Gln	Gln	Gln	
				980					985					990			
5	CTA	TTA	CGT	CTG	GTG	AGA	CAA	AAA	AAT	AGC	TGG	CAT	CAC	CTG	ACT	GAT	3024
	Leu	Leu	Arg	Leu	Val	Arg	Gln	Lys	Asn	Ser	Trp	His	His	Leu	Thr	Asp	
			995					1000					1005				
10	GGG	GAA	AAC	TGG	CGA	TTA	GGT	TTA	CCG	AAT	GCA	CAA	CGC	CGT	GAT	GTT	3072
	Gly	Glu	Asn	Trp	Arg	Leu	Gly	Leu	Pro	Asn	Ala	Gln	Arg	Arg	Asp	Val	
		1010					1015					1020					
15	TAT	ACT	TAT	GAC	CGG	AGC	AAA	ATT	CCA	ACC	GAA	GGG	ATT	TCC	CTT	GAA	3120
	Tyr	Thr	Tyr	Asp	Arg	Ser	Lys	Ile	Pro	Thr	Glu	Gly	Ile	Ser	Leu	Glu	
	1025						1030				1035					1040	
20	ATC	TTG	CTG	AAA	GAT	GAT	GGC	CTG	CTA	GCA	GAT	GAA	AAA	GCG	GCC	GTT	3168
	Ile	Leu	Leu	Lys	Asp	Asp	Gly	Leu	Leu	Ala	Asp	Glu	Lys	Ala	Ala	Val	
					1045					1050					1055		
	TAT	CTG	GGA	CAA	CAA	CAG	ACG	TTT	TAC	ACC	GCC	GGT	CAA	GCG	GAA	GTC	3216
	Tyr	Leu	Gly	Gln	Gln	Gln	Thr	Phe	Tyr	Thr	Ala	Gly	Gln	Ala	Glu	Val	
				1060				1065						1070			
25	ACT	CTA	GAA	AAA	CCC	ACG	TTA	CAA	GCA	CTG	GTC	GCG	TTC	CAA	GAA	ACC	3264
	Thr	Leu	Glu	Lys	Pro	Thr	Leu	Gln	Ala	Leu	Val	Ala	Phe	Gln	Glu	Thr	
			1075					1080					1085				
30	GCC	ATG	ATG	GAC	GAT	ACC	TCA	TTA	CAG	GCG	TAT	GAA	GGC	GTG	ATT	GAA	3312
	Ala	Met	Met	Asp	Asp	Thr	Ser	Leu	Gln	Ala	Tyr	Glu	Gly	Val	Ile	Glu	
		1090					1095					1100					
35	GAG	CAA	GAG	TTG	AAT	ACC	GCG	CTG	ACA	CAG	GCC	GGT	TAT	CAG	CAA	GTC	3360
	Glu	Gln	Glu	Leu	Asn	Thr	Ala	Leu	Thr	Gln	Ala	Gly	Tyr	Gln	Gln	Val	
	1105				1110					1115						1120	
40	GCG	CGG	TTG	TTT	AAT	ACC	AGA	TCA	GAA	AGC	CCG	GTA	TGG	GCG	GCA	CGG	3408
	Ala	Arg	Leu	Phe	Asn	Thr	Arg	Ser	Glu	Ser	Pro	Val	Trp	Ala	Ala	Arg	
				1125				1130							1135		
	CAA	GGT	TAT	ACC	GAT	TAC	GGT	GAC	GCC	GCA	CAG	TTC	TGG	CGG	CCT	CAG	3456
	Gln	Gly	Tyr	Thr	Asp	Tyr	Gly	Asp	Ala	Ala	Gln	Phe	Trp	Arg	Pro	Gln	
			1140				1145							1150			
45	GCT	CAG	CGT	AAC	TCG	TTG	CTG	ACA	GGG	AAA	ACC	ACA	CTG	ACC	TGG	GAT	3504
	Ala	Gln	Arg	Asn	Ser	Leu	Leu	Thr	Gly	Lys	Thr	Thr	Leu	Thr	Trp	Asp	
			1155				1160						1165				
50	ACC	CAT	CAT	TGT	GTA	ATA	ATA	CAG	ACT	CAA	GAT	GCC	GCT	GGA	TTA	ACG	3552
	Thr	His	His	Cys	Val	Ile	Ile	Gln	Thr	Gln	Asp	Ala	Ala	Gly	Leu	Thr	
		1170				1175					1180						
55	ACG	CAA	GCC	CAT	TAC	GAT	TAT	CGT	TTC	CTT	ACA	CCG	GTA	CAA	CTG	ACA	3600
	Thr	Gln	Ala	His	Tyr	Asp	Tyr	Arg	Phe	Leu	Thr	Pro	Val	Gln	Leu	Thr	
	1185					1190				1195					1200		
60	GAT	ATT	AAT	GAT	AAT	CAA	CAT	ATT	GTG	ACT	CTG	GAC	GCG	CTA	GGT	CGC	3648
	Asp	Ile	Asn	Asp	Asn	Gln	His	Ile	Val	Thr	Leu	Asp	Ala	Leu	Gly	Arg	
				1205				1210						1215			
	GTA	ACC	ACC	AGC	CGG	TTC	TGG	GGC	ACA	GAG	GCA	GGA	CAA	GCC	GCA	GGC	3696
	Val	Thr	Thr	Ser	Arg	Phe	Trp	Gly	Thr	Glu	Ala	Gly	Gln	Ala	Ala	Gly	
				1220				1225						1230			
65	TAT	TCC	AAC	CAG	CCC	TTC	ACA	CCA	CCG	GAC	TCC	GTA	GAT	AAA	GCG	CTG	3744
	Tyr	Ser	Asn	Gln	Pro	Phe	Thr	Pro	Pro	Asp	Ser	Val	Asp	Lys	Ala	Leu	

	1235	1240	1245	
5	GCA TTA ACC GGC GCA CTC CCT GTT GCC CAA TGT TTA GTC TAT GCC GTT 3732 Ala Leu Thr Gly Ala Leu Pro Val Ala Gln Cys Leu Val Tyr Ala Val 1250 1255 1260			
10	GAT AGC TGG ATG CCG TCG TTA TCT TTG TCT CAG CTT TCT CAG TCA CAA 3840 Asp Ser Trp Met Pro Ser Leu Ser Leu Ser Gln Leu Ser Gln Ser Gln 1265 1270 1275 1280			
	GAA GAG GCA GAA GCG CTA TGG GCG CAA CTG CGT GCC GCT CAT ATG ATT 3888 Glu Glu Ala Glu Ala Leu Trp Ala Gln Leu Arg Ala Ala His Met Ile 1285 1290 1295			
15	ACC GAA GAT GGG AAA GTG TGT GCG TTA AGC GGG AAA CGA GGA ACA AGC 3936 Thr Glu Asp Gly Lys Val Cys Ala Leu Ser Gly Lys Arg Gly Thr Ser 1300 1305 1310			
20	CAT CAG AAC CTG ACG ATT CAA CTT ATT TCG CTA TTG GCA AGT ATT CCC 3984 His Gln Asn Leu Thr Ile Gln Leu Ile Ser Leu Leu Ala Ser Ile Pro 1315 1320 1325			
25	CGT TTA CCG CCA CAT GTA CTG GGG ATC ACC ACT GAT CGC TAT GAT AGC 4032 Arg Leu Pro Pro His Val Leu Gly Ile Thr Thr Asp Arg Tyr Asp Ser 1330 1335 1340			
30	GAT CCG CAA CAG CAG CAC CAA CAG ACG GTG AGC TTT AGT GAC GGT TTT 4080 Asp Pro Gln Gln Gln His Gln Gln Thr Val Ser Phe Ser Asp Gly Phe 1345 1350 1355 1360			
	GGC CGG TTA CTC CAG AGT TCA GCT CGT CAT GAG TCA GGT GAT GCC TGG 4128 Gly Arg Leu Leu Gln Ser Ser Ala Arg His Glu Ser Gly Asp Ala Trp 1365 1370 1375			
35	CAA CGT AAA GAG GAT GGC GGG CTG GTC GTG GAT GCA AAT GGC GTT CTG 4176 Gln Arg Lys Glu Asp Gly Gly Leu Val Val Asp Ala Asn Gly Val Leu 1380 1385 1390			
40	GTC AGT GCC CCT ACA GAC ACC CGA TGG GCC GTT TCC GGT CGC ACA GAA 4224 Val Ser Ala Pro Thr Asp Thr Arg Trp Ala Val Ser Gly Arg Thr Glu 1395 1400 1405			
45	TAT GAC GAC AAA GGC CAA CCT GTG CGT ACT TAT CAA CCC TAT TTT CTA 4272 Tyr Asp Asp Lys Gly Gln Pro Val Arg Thr Tyr Gln Pro Tyr Phe Leu 1410 1415 1420			
50	AAT GAC TGG CGT TAC GTT AGT GAT GAC AGC GCA CGA GAT GAC CTG TTT 4320 Asn Asp Trp Arg Tyr Val Ser Asp Asp Ser Ala Arg Asp Asp Leu Phe 1425 1430 1435 1440			
	GCC GAT ACC CAC CTT TAT GAT CCA TTG GGA CGG GAA TAC AAA GTC ATC 4368 Ala Asp Thr His Leu Tyr Asp Pro Leu Gly Arg Glu Tyr Lys Val Ile 1445 1450 1455			
55	ACT GCT AAG AAA TAT TTG CGA GAA AAG CTG TAC ACC CCG TGG TTT ATT 4416 Thr Ala Lys Lys Tyr Leu Arg Glu Lys Leu Tyr Thr Pro Trp Phe Ile 1460 1465 1470			
60	GTC AGT GAG GAT GAA AAC GAT ACA GCA TCA AGA ACC CCA TAG 4458 Val Ser Glu Asp Glu Asn Asp Thr Ala Ser Arg Thr Pro * 1475 1480 1485			

65 (2) INFORMATION FOR SEQ ID NO:32:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1486 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

10

Met Gln Asp Ser Pro Glu Val Ser Ile Thr Thr Leu Ser Leu Pro Lys  
 1 5 10 15

15

Gly Gly Gly Ala Ile Asn Gly Met Gly Glu Ala Leu Asn Ala Ala Gly  
 20 25 30

Pro Asp Gly Met Ala Ser Leu Ser Leu Pro Leu Pro Leu Ser Thr Gly  
 35 40 45

20

Arg Gly Thr Ala Pro Gly Leu Ser Leu Ile Tyr Ser Asn Ser Ala Gly  
 50 55 60

Asn Gly Pro Phe Gly Ile Gly Trp Gln Cys Gly Val Met Ser Ile Ser  
 65 70 75 80

25

Arg Arg Thr Gln His Gly Ile Pro Gln Tyr Gly Asn Asp Asp Thr Phe  
 85 90 95

30

Leu Ser Pro Gln Gly Glu Val Met Asn Ile Ala Leu Asn Asp Gln Gly  
 100 105 110

Gln Pro Asp Ile Arg Gln Asp Val Lys Thr Leu Gln Gly Val Thr Leu  
 115 120 125

35

Pro Ile Ser Tyr Thr Val Thr Arg Tyr Gln Ala Arg Gln Ile Leu Asp  
 130 135 140

Phe Ser Lys Ile Glu Tyr Trp Gln Pro Ala Ser Gly Gln Glu Gly Arg  
 145 150 155 160

40

Ala Phe Trp Leu Ile Ser Thr Pro Asp Gly His Leu His Ile Leu Gly  
 165 170 175

Lys Thr Ala Gln Ala Cys Leu Ala Asn Pro Gln Asn Asp Gln Gln Ile  
 180 185 190

45

Ala Gln Trp Leu Leu Glu Glu Thr Val Thr Pro Ala Gly Glu His Val  
 195 200 205

50

Ser Tyr Gln Tyr Arg Ala Glu Asp Glu Ala His Cys Asp Asp Asn Glu  
 210 215 220

Lys Thr Ala His Pro Asn Val Thr Ala Gln Arg Tyr Leu Val Gln Val  
 225 230 235 240

55

Asn Tyr Gly Asn Ile Lys Pro Gln Ala Ser Leu Phe Val Leu Asp Asn  
 245 250 255

Ala Pro Pro Ala Pro Glu Glu Trp Leu Phe His Leu Val Phe Asp His  
 260 265 270

60

Gly Glu Arg Asp Thr Ser Leu His Thr Val Pro Thr Trp Asp Ala Gly  
 275 280 285

65

Thr Ala Gln Trp Ser Val Arg Pro Asp Ile Phe Ser Arg Tyr Glu Tyr

	290		295		300
	Gly Phe Glu Val Arg Thr Arg Arg Leu Cys Gln Gln Val Leu Met Phe				
	305		310		315 320
5	His Arg Thr Ala Leu Met Ala Gly Glu Ala Ser Thr Asn Asp Ala Pro				
		325		330	335
10	Glu Leu Val Gly Arg Leu Ile Leu Glu Tyr Asp Lys Asn Ala Ser Val				
		340		345	350
	Thr Thr Leu Ile Thr Ile Arg Gln Leu Ser His Glu Ser Asp Gly Arg				
		355		360	365
15	Pro Val Thr Gln Pro Pro Leu Glu Leu Ala Trp Gln Arg Phe Asp Leu				
		370		375	380
	Glu Lys Ile Pro Thr Trp Gln Arg Phe Asp Ala Leu Asp Asn Phe Asn				
	385		390		395 400
20	Ser Gln Gln Arg Tyr Gln Leu Val Asp Leu Arg Gly Glu Gly Leu Pro				
		405		410	415
25	Gly Met Leu Tyr Gln Asp Arg Gly Ala Trp Trp Tyr Lys Ala Pro Gln				
		420		425	430
	Arg Gln Glu Asp Gly Asp Ser Asn Ala Val Thr Tyr Asp Lys Ile Ala				
		435		440	445
30	Pro Leu Pro Thr Leu Pro Asn Leu Gln Asp Asn Ala Ser Leu Met Asp				
		450		455	460
	Ile Asn Gly Asp Gly Gln Leu Asp Trp Val Val Thr Ala Ser Gly Ile				
	465		470		475 480
35	Arg Gly Tyr His Ser Gln Gln Pro Asp Gly Lys Trp Thr His Phe Thr				
		485		490	495
	Pro Ile Asn Ala Leu Pro Val Glu Tyr Phe His Pro Ser Ile Gln Phe				
40		500		505	510
	Ala Asp Leu Thr Gly Ala Gly Leu Ser Asp Leu Val Leu Ile Gly Pro				
		515		520	525
45	Lys Ser Val Arg Leu Tyr Ala Asn Gln Arg Asn Gly Trp Arg Lys Gly				
		530		535	540
	Glu Asp Val Pro Gln Ser Thr Gly Ile Thr Leu Pro Val Thr Gly Thr				
	545		550		555 560
50	Asp Ala Arg Lys Leu Val Ala Phe Ser Asp Met Leu Gly Ser Gly Gln				
		565		570	575
	Gln His Leu Val Glu Ile Lys Gly Asn Arg Val Thr Cys Trp Pro Asn				
55		580		585	590
	Leu Gly His Gly Arg Phe Gly Gln Pro Leu Thr Leu Ser Gly Phe Ser				
		595		600	605
60	Gln Pro Glu Asn Ser Phe Asn Pro Glu Arg Leu Phe Leu Ala Asp Ile				
		610		615	620
	Asp Gly Ser Gly Thr Thr Asp Leu Ile Tyr Ala Gln Ser Gly Ser Leu				
	625		630		635 640
65	Leu Ile Tyr Leu Asn Gln Ser Gly Asn Gln Phe Asp Ala Pro Leu Thr				

				645				650					655				
				Leu	Ala	Leu	Pro	Glu	Gly	Val	Gln	Phe	Asp	Asn	Thr	Cys	
				650								665				Gln	
5				Val	Ala	Asp	Ile	Gln	Gly	Leu	Gly	Ile	Ala	Ser	Leu	Ile	Leu
				675							680				685	Thr	
				Pro	His	Ile	Ala	Pro	His	His	Trp	Arg	Cys	Asp	Leu	Ser	Leu
10				690						695					700	Thr	Lys
				Pro	Trp	Leu	Leu	Asn	Val	Met	Asn	Asn	Asn	Arg	Gly	Ala	His
				705				710						715		His	Thr
15				Leu	His	Tyr	Arg	Ser	Ala	Gln	Phe	Trp	Leu	Asp	Glu	Lys	Leu
				725								730				735	Gln
				Leu	Thr	Lys	Ala	Gly	Lys	Ser	Pro	Ala	Cys	Tyr	Leu	Pro	Phe
20				740							745					750	Pro
				His	Leu	Leu	Trp	Tyr	Thr	Glu	Ile	Gln	Asp	Glu	Ile	Ser	Gly
				755						760					765	Asn	Arg
25				Leu	Thr	Ser	Glu	Val	Asn	Tyr	Ser	His	Gly	Val	Trp	Asp	Gly
				770					775						780	Lys	Glu
				Arg	Glu	Phe	Arg	Gly	Phe	Gly	Cys	Ile	Lys	Gln	Thr	Asp	Thr
				785				790						795			Thr
30				Phe	Ser	His	Gly	Thr	Ala	Pro	Glu	Gln	Ala	Ala	Pro	Ser	Leu
				805									810				Ser
				Ser	Trp	Phe	Ala	Thr	Gly	Met	Asp	Glu	Val	Asp	Ser	Gln	Leu
35				820							825					830	Ala
				Glu	Tyr	Trp	Gln	Ala	Asp	Thr	Gln	Ala	Tyr	Ser	Gly	Phe	Glu
				835						840					845	Thr	Arg
40				Tyr	Thr	Val	Trp	Asp	His	Thr	Asn	Gln	Thr	Asp	Gln	Ala	Phe
				850						855					860	Thr	Pro
				Asn	Glu	Thr	Gln	Arg	Asn	Trp	Leu	Thr	Arg	Ala	Leu	Lys	Gly
				865				870						875			Gln
45				Leu	Arg	Thr	Glu	Leu	Tyr	Gly	Leu	Asp	Gly	Thr	Asp	Lys	Gln
				885								890					Thr
				Pro	Tyr	Thr	Val	Ser	Glu	Ser	Arg	Tyr	Gln	Val	Arg	Ser	Ile
50				900							905					910	Pro
				Asn	Lys	Glu	Thr	Glu	Leu	Ser	Ala	Trp	Val	Thr	Ala	Ile	Glu
				915						920					925	Asn	Arg
55				Ser	Tyr	His	Tyr	Glu	Arg	Ile	Ile	Thr	Asp	Pro	Gln	Phe	Ser
				930						935					940	Gln	Ser
				Ile	Lys	Leu	Gln	His	Asp	Ile	Phe	Gly	Gln	Ser	Leu	Gln	Ser
				945				950						955			Val
60				Ile	Ala	Trp	Pro	Arg	Arg	Glu	Lys	Pro	Ala	Val	Asn	Pro	Tyr
				965									970				Pro
				Thr	Leu	Pro	Glu	Thr	Leu	Phe	Asp	Ser	Ser	Tyr	Asp	Asp	Gln
65				980							985					990	Gln
				Leu	Leu	Arg	Leu	Val	Arg	Gln	Lys	Asn	Ser	Trp	His	His	Leu
																Thr	Asp

	995	1000	1005
	Gly Glu Asn Trp Arg Leu	Gly Leu Pro Asn Ala	Gln Arg Arg Asp Val
	1010	1015	1020
5	Tyr Thr Tyr Asp Arg Ser Lys Ile Pro Thr	Glu Gly Ile Ser Leu Glu	
	1025	1030	1035 1040
	Ile Leu Leu Lys Asp Asp Gly Leu Leu Ala Asp Glu Lys Ala Ala Val		
10		1045	1050 1055
	Tyr Leu Gly Gln Gln Gln Thr Phe Tyr Thr Ala Gly Gln Ala Glu Val		
		1060	1065 1070
15	Thr Leu Glu Lys Pro Thr Leu Gln Ala Leu Val Ala Phe Gln Glu Thr		
		1075	1080 1085
	Ala Met Met Asp Asp Thr Ser Leu Gln Ala Tyr Glu Gly Val Ile Glu		
20		1090	1095 1100
	Glu Gln Glu Leu Asn Thr Ala Leu Thr Gln Ala Gly Tyr Gln Gln Val		
		1105	1110 1115 1120
	Ala Arg Leu Phe Asn Thr Arg Ser Glu Ser Pro Val Trp Ala Ala Arg		
25		1125	1130 1135
	Gln Gly Tyr Thr Asp Tyr Gly Asp Ala Ala Gln Phe Trp Arg Pro Gln		
		1140	1145 1150
30	Ala Gln Arg Asn Ser Leu Leu Thr Gly Lys Thr Thr Leu Thr Trp Asp		
		1155	1160 1165
	Thr His His Cys Val Ile Ile Gln Thr Gln Asp Ala Ala Gly Leu Thr		
35		1170	1175 1180
	Thr Gln Ala His Tyr Asp Tyr Arg Phe Leu Thr Pro Val Gln Leu Thr		
		1185	1190 1195 1200
	Asp Ile Asn Asp Asn Gln His Ile Val Thr Leu Asp Ala Leu Gly Arg		
40		1205	1210 1215
	Val Thr Thr Ser Arg Phe Trp Gly Thr Glu Ala Gly Gln Ala Ala Gly		
		1220	1225 1230
45	Tyr Ser Asn Gln Pro Phe Thr Pro Pro Asp Ser Val Asp Lys Ala Leu		
		1235	1240 1245
	Ala Leu Thr Gly Ala Leu Pro Val Ala Gln Cys Leu Val Tyr Ala Val		
50		1250	1255 1260
	Asp Ser Trp Met Pro Ser Leu Ser Leu Ser Gln Leu Ser Gln Ser Gln		
		1265	1270 1275 1280
	Glu Glu Ala Glu Ala Leu Trp Ala Gln Leu Arg Ala Ala His Met Ile		
55		1285	1290 1295
	Thr Glu Asp Gly Lys Val Cys Ala Leu Ser Gly Lys Arg Gly Thr Ser		
		1300	1305 1310
60	His Gln Asn Leu Thr Ile Gln Leu Ile Ser Leu Leu Ala Ser Ile Pro		
		1315	1320 1325
	Arg Leu Pro Pro His Val Leu Gly Ile Thr Thr Asp Arg Tyr Asp Ser		
65		1330	1335 1340
	Asp Pro Gln Gln Gln His Gln Gln Thr Val Ser Phe Ser Asp Gly Phe		

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1345          1350          1355          1360
Gly Arg Leu Leu Gln Ser Ser Ala Arg His Glu Ser Gly Asp Ala Trp
                    1365                    1370                    1375
5  Gln Arg Lys Glu Asp Gly Gly Leu Val Val Asp Ala Asn Gly Val Leu
                    1380                    1385                    1390
10 Val Ser Ala Pro Thr Asp Thr Arg Trp Ala Val Ser Gly Arg Thr Glu
                    1395                    1400                    1405
Tyr Asp Asp Lys Gly Gln Pro Val Arg Thr Tyr Gln Pro Tyr Phe Leu
                    1410                    1415                    1420
15 Asn Asp Trp Arg Tyr Val Ser Asp Asp Ser Ala Arg Asp Asp Leu Phe
                    1425                    1430                    1435                    1440
Ala Asp Thr His Leu Tyr Asp Pro Leu Gly Arg Glu Tyr Lys Val Ile
                    1445                    1450                    1455
20 Thr Ala Lys Lys Tyr Leu Arg Glu Lys Leu Tyr Thr Pro Trp Phe Ile
                    1460                    1465                    1470
25 Val Ser Glu Asp Glu Asn Asp Thr Ala Ser Arg Thr Pro *
                    1475                    1480                    1485

```

## (2) INFORMATION FOR SEQ ID NO:33:

```

30 (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 3288 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: double
      (D) TOPOLOGY: linear
35 (ii) MOLECULE TYPE: DNA (genomic)

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

```

40 ATG GTG ACT GTT ATG CAA AAT AAA ATA TCA TTT TTA TCA GGT ACA TCC 48
   Met Val Thr Val Met Gln Asn Lys Ile Ser Phe Leu Ser Gly Thr Ser
     1             5             10             15
45 GAA CAG CCC CTG CTT GAC GCC GGT TAT CAA AAC GTA TTT GAT ATC GCA 96
   Glu Gln Pro Leu Leu Asp Ala Gly Tyr Gln Asn Val Phe Asp Ile Ala
           20             25             30
50 TCA ATC AGC CGG GCT ACT TTC GTT CAA TCC GTT CCC ACC CTG CCC GTT 144
   Ser Ile Ser Arg Ala Thr Phe Val Gln Ser Val Pro Thr Leu Pro Val
           35             40             45
55 AAA GAG GCT CAT ACC GTC TAT CGT CAG GCG CGG CAA CGT GCG GAA AAT 192
   Lys Glu Ala His Thr Val Tyr Arg Gln Ala Arg Gln Arg Ala Glu Asn
           50             55             60
60 CTG AAA TCC CTC TAC CGA GCC TGG CAA TTG CGT CAG GAG CCG GTT ATT 240
   Leu Lys Ser Leu Tyr Arg Ala Trp Gln Leu Arg Gln Glu Pro Val Ile
           65             70             75             80
65 AAA GGG CTG GCT AAA CTT AAC CTA CAA TCC AAC GTT TCT GTG CTT CAA 238
   Lys Gly Leu Ala Lys Leu Asn Leu Gln Ser Asn Val Ser Val Leu Gln
           85             90             95
70 GAT GCT TTG GTA GAG AAT ATT GGC GGT GAT GGG GAT TTC AGC GAT TTA 336
   Asp Ala Leu Val Glu Asn Ile Gly Gly Asp Gly Asp Phe Ser Asp Leu

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		100					105						110				
	5	ATG AAC CGT GCC AGT CAA TAT GCT GAC GCT GCC TCT ATT CAA TCC CTA	334	Met Asn Arg Ala Ser Gln Tyr Ala Asp Ala Ala Ser Ile Gln Ser Leu													
			115				120						125				
	10	TTT TCA CCG GGC CGT TAT GCT TCC GCA CTC TAC AGA GTT GCT AAA GAT	432	Phe Ser Pro Gly Arg Tyr Ala Ser Ala Leu Tyr Arg Val Ala Lys Asp													
			130				135						140				
	15	CTG CAT AAA TCA GAT TCC AGT TTG CAT ATT GAT AAT CGC CGC GCT GAT	480	Leu His Lys Ser Asp Ser Ser Leu His Ile Asp Asn Arg Arg Ala Asp													
			145				150						155				160
	20	CTG AAG GAT CTG ATA TTA AGC GAA ACG ACG ATG AAT AAA GAG GTC ACT	528	Leu Lys Asp Leu Ile Leu Ser Glu Thr Thr Met Asn Lys Glu Val Thr													
							165										175
	25	TCC CTT GAT ATC TTG TTG GAT GTG CTA CAA AAA GGC GGT AAA GAT ATT	576	Ser Leu Asp Ile Leu Leu Asp Val Leu Gln Lys Gly Gly Lys Asp Ile													
							180										190
	30	ACT GAG CTG TCC GGC GCA TTC TTC CCA ATG ACG TTA CCT TAT GAC GAT	624	Thr Glu Leu Ser Gly Ala Phe Phe Pro Met Thr Leu Pro Tyr Asp Asp													
			195				200						205				
	35	CAT CTG TCG CAA ATC GAT TCC GCT TTA TCG GCA CAA GCC AGA ACG CTG	672	His Leu Ser Gln Ile Asp Ser Ala Leu Ser Ala Gln Ala Arg Thr Leu													
			210				215						220				
	40	AAC GGT GTG TGG AAT ACT TTG ACA GAT ACC ACG GCA CAA GCG GTT TCA	720	Asn Gly Val Trp Asn Thr Leu Thr Asp Thr Thr Ala Gln Ala Val Ser													
			225				230						235				240
	45	GAA CAA ACC AGT AAT ACG AAT ACA CGC AAA CTG TTC GCT GCC CAA GAT	768	Glu Gln Thr Ser Asn Thr Asn Thr Arg Lys Leu Phe Ala Ala Gln Asp													
							245										255
	50	GGT AAT CAA GAT ACA TTT TTT TCC GGA AAC ACT TTT TAT TTC AAA GCG	816	Gly Asn Gln Asp Thr Phe Phe Ser Gly Asn Thr Phe Tyr Phe Lys Ala													
			260				265										270
	55	GTG GGA TTC AGC GGG CAA CCT ATG GTT TAC CTG TCA CAG TAC ACC AGC	864	Val Gly Phe Ser Gly Gln Pro Met Val Tyr Leu Ser Gln Tyr Thr Ser													
			275				280						285				
	60	GGG AAC GGC ATT GTC GGC GCA CAA TTG ATT GCA GGT AAT CCA GAC CAA	912	Gly Asn Gly Ile Val Gly Ala Gln Leu Ile Ala Gly Asn Pro Asp Gln													
			290				295						300				
	65	GCC GCC GCC GCA ATA GTC GCA CCG TTG AAA CTC ACT TGG TCA ATG GCA	960	Ala Ala Ala Ala Ile Val Ala Pro Leu Lys Leu Thr Trp Ser Met Ala													
			305				310						315				320
	70	AAA CAG TGT TAC TAC CTC GTC GCT CCC GAT GGT ACA ACG ATG GGA GAC	1008	Lys Gln Cys Tyr Tyr Leu Val Ala Pro Asp Gly Thr Thr Met Gly Asp													
							325										335
	75	GGT AAT GTT CTG ACC GGC TGT TTC TTA AGA GGC AAC AGC CCA ACT AAC	1056	Gly Asn Val Leu Thr Gly Cys Phe Leu Arg Gly Asn Ser Pro Thr Asn													
			340				345										350
	80	CCG GAT AAA GAC GGT ATT TTT GCT CAG GTA GCC AAC AAA TCA GGC AGT	1104	Pro Asp Lys Asp Gly Ile Phe Ala Gln Val Ala Asn Lys Ser Gly Ser													
			355				360										365

	ACT	CAG	CCT	TTG	CCA	AGC	TTC	CAT	CTG	CCG	GTC	ACA	CTG	GAA	CAC	AGC	1152
	Thr	Gln	Pro	Leu	Pro	Ser	Phe	His	Leu	Pro	Val	Thr	Leu	Glu	His	Ser	
	370						375					380					
5	GAG	AAT	AAA	GAT	CAG	TAC	TAT	CTG	AAA	ACA	GAG	CAG	GGT	TAT	ATC	ACG	1200
	Glu	Asn	Lys	Asp	Gln	Tyr	Tyr	Leu	Lys	Thr	Glu	Gln	Gly	Tyr	Ile	Thr	
	385					390					395					400	
10	GTA	GAT	AGT	TCC	GGA	CAG	TCA	AAT	TGG	AAA	AAC	GCG	CTG	GTT	ATC	AAT	1248
	Val	Asp	Ser	Ser	Gly	Gln	Ser	Asn	Trp	Lys	Asn	Ala	Leu	Val	Ile	Asn	
					405					410					415		
15	GGG	ACA	AAA	GAC	AAG	GGG	CTG	TTA	TTA	ACC	TTT	TGC	AGC	GAT	AGC	TCA	1296
	Gly	Thr	Lys	Asp	Lys	Gly	Leu	Leu	Leu	Thr	Phe	Cys	Ser	Asp	Ser	Ser	
				420				425						430			
20	GGC	ACT	CCG	ACA	AAC	CCT	GAT	GAT	GTG	ATT	CCT	CCC	GCT	ATC	AAT	GAT	1344
	Gly	Thr	Pro	Thr	Asn	Pro	Asp	Asp	Val	Ile	Pro	Pro	Ala	Ile	Asn	Asp	
				435				440					445				
25	ATT	CCA	TCG	CCG	CCA	GCC	CGC	GAA	ACA	CTG	TCA	CTG	ACG	CCG	GTC	AGT	1392
	Ile	Pro	Ser	Pro	Pro	Ala	Arg	Glu	Thr	Leu	Ser	Leu	Thr	Pro	Val	Ser	
				450			455					460					
30	TAT	CAA	TTG	ATG	ACC	AAT	CCG	GCA	CCG	ACA	GAA	GAT	GAT	ATT	ACC	AAC	1440
	Tyr	Gln	Leu	Met	Thr	Asn	Pro	Ala	Pro	Thr	Glu	Asp	Asp	Ile	Thr	Asn	
	465					470					475					480	
35	CAT	TAT	GGT	TTT	AAC	GGC	GCT	AGC	TTA	CGG	GCT	TCT	CCA	TTG	TCA	ACC	1488
	His	Tyr	Gly	Phe	Asn	Gly	Ala	Ser	Leu	Arg	Ala	Ser	Pro	Leu	Ser	Thr	
					485					490					495		
40	AGC	GAG	TTG	ACC	AGC	AAA	CTG	AAT	TCT	ATC	GAT	ACT	TTC	TGT	GAG	AAG	1536
	Ser	Glu	Leu	Thr	Ser	Lys	Leu	Asn	Ser	Ile	Asp	Thr	Phe	Cys	Glu	Lys	
				500					505					510			
45	ACC	CGG	TTA	AGC	TTC	AAT	CAG	TTA	ATG	GAT	TTG	ACC	GCT	CAG	CAA	TCT	1584
	Thr	Arg	Leu	Ser	Phe	Asn	Gln	Leu	Met	Asp	Leu	Thr	Ala	Gln	Gln	Ser	
				515				520					525				
50	TAC	AGT	CAA	AGC	AGC	ATT	GAT	GCG	AAA	GCA	GCC	AGC	CGC	TAT	GTT	CGT	1632
	Tyr	Ser	Gln	Ser	Ser	Ile	Asp	Ala	Lys	Ala	Ala	Ser	Arg	Tyr	Val	Arg	
		530					535					540					
55	TTT	GGG	GAA	ACC	ACC	CCA	ACC	CGC	GTC	AAT	GTC	TAC	GGT	GCC	GCT	TAT	1680
	Phe	Gly	Glu	Thr	Thr	Pro	Thr	Arg	Val	Asn	Val	Tyr	Gly	Ala	Ala	Tyr	
	545					550				555						560	
60	CTG	AAC	AGC	ACA	CTG	GCA	GAC	GCG	GCT	GAT	GGT	CAA	TAT	CTG	TGG	ATT	1728
	Leu	Asn	Ser	Thr	Leu	Ala	Asp	Ala	Ala	Asp	Gly	Gln	Tyr	Leu	Trp	Ile	
					565					570					575		
65	CAG	ACT	GAT	GGC	AAG	AGC	CTA	AAT	TTC	ACT	GAC	GAT	ACG	GTA	GTC	GCC	1776
	Gln	Thr	Asp	Gly	Lys	Ser	Leu	Asn	Phe	Thr	Asp	Asp	Thr	Val	Val	Ala	
				580					585					590			
70	TTA	GCC	GGT	CGC	GCT	GAA	AAG	CTG	GTA	CGT	TTA	TCA	TCC	CAG	ACC	GGG	1824
	Leu	Ala	Gly	Arg	Ala	Glu	Lys	Leu	Val	Arg	Leu	Ser	Ser	Gln	Thr	Gly	
			595					600					605				
75	CTA	TCA	TTT	GAA	GAA	TTG	GAC	TGG	CTG	ATT	GCC	AAT	GCC	AGT	CGT	AGT	1872
	Leu	Ser	Phe	Glu	Glu	Leu	Asp	Trp	Leu	Ile	Ala	Asn	Ala	Ser	Arg	Ser	
			610				615					620					
80	GTG	CCG	GAC	CAC	CAC	GAC	AAA	ATT	GTG	CTG	GAT	AAG	CCG	GTC	CTT	GAA	1920
	Val	Pro	Asp	His	His	Asp	Lys	Ile	Val	Leu	Asp	Lys	Pro	Val	Leu	Glu	

	625		630		635		640	
5	GCA CTG GCA GAG TAT GTC AGC CTA AAA CAG CGC TAT GGG CTT GAT GCC 1963 Ala Leu Ala Glu Tyr Val Ser Leu Lys Gln Arg Tyr Gly Leu Asp Ala 655	645		650				
10	AAT ACC TTT GCG ACC TTC ATT AGT GCA GTA AAT CCT TAT ACG CCA GAT 2015 Asn Thr Phe Ala Thr Phe Ile Ser Ala Val Asn Pro Tyr Thr Pro Asp 660 665 670	660		665				
15	CAG ACA CCC AGT TTC TAT GAA ACC GCT TTC CGC TCT GCC GAC GGT AAT 2064 Gln Thr Pro Ser Phe Tyr Glu Thr Ala Phe Arg Ser Ala Asp Gly Asn 675 680 685	675		680				
20	CAT GTC ATT GCG CTA GGT ACA GAG GTG AAA TAT GCA GAA AAT GAG CAG 2112 His Val Ile Ala Leu Gly Thr Glu Val Lys Tyr Ala Glu Asn Glu Gln 690 695 700	690		695				
25	GAT GAG TTA GCC GCC ATA TGC TGC AAA GCA TTG GGT GTC ACC AGT GAT 2160 Asp Glu Leu Ala Ala Ile Cys Cys Lys Ala Leu Gly Val Thr Ser Asp 705 710 715 720	705		710				
30	GAA CTG CTC CGT ATT GGT CGC TAT TGC TTC GGT AAT GCA GGC AGT TTT 2208 Glu Leu Leu Arg Ile Gly Arg Tyr Cys Phe Gly Asn Ala Gly Ser Phe 725 730 735	725		730				
35	ACC TTG GAT GAA TAT ACC GCC AGT CAG TTG TAT CGC TTC GGC GCC ATT 2256 Thr Leu Asp Glu Tyr Thr Ala Ser Gln Leu Tyr Arg Phe Gly Ala Ile 740 745 750	740		745				
40	CCC CGT TTG TTT GGG CTG ACA TTT GCC CAA GCC GAA ATT TTA TGG CGT 2304 Pro Arg Leu Phe Gly Leu Thr Phe Ala Gln Ala Glu Ile Leu Trp Arg 755 760 765	755		760				
45	CTG ATG GAA GGC GGA AAA GAT ATC TTA TTG CAA CAG TTA GGT CAG GCA 2352 Leu Met Glu Gly Gly Lys Asp Ile Leu Leu Gln Gln Leu Gly Gln Ala 770 775 780	770		775				
50	AAA TCC CTG CAA CCA CTG GCT ATT TTA CGC CGT ACC GAG CAG GTG CTG 2400 Lys Ser Leu Gln Pro Leu Ala Ile Leu Arg Arg Thr Glu Gln Val Leu 785 790 795 800	785		790				
55	GAT TGG ATG TCG TCC GTA AAT CTA AGT CTG ACT TAT CTG CAA GGG ATG 2448 Asp Trp Met Ser Ser Val Asn Leu Ser Leu Thr Tyr Leu Gln Gly Met 805 810 815	805		810				
60	GTA AGT ACG CAA TGG AGC GGT ACC GCC ACC GCT GAG ATG TTC AAT TTC 2496 Val Ser Thr Gln Trp Ser Gly Thr Ala Thr Ala Glu Met Phe Asn Phe 820 825 830	820		825				
65	TTG GAA AAC GTT TGT GAC AGC GTG AAT AGT CAA GCT GCC ACT AAA GAA 2544 Leu Glu Asn Val Cys Asp Ser Val Asn Ser Gln Ala Ala Thr Lys Glu 835 840 845	835		840				
70	ACA ATG GAT TCG GCG TTA CAG CAG AAA GTG CTG CGG GCG CTA AGC GCC 2592 Thr Met Asp Ser Ala Leu Gln Gln Lys Val Leu Arg Ala Leu Ser Ala 850 855 860	850		855				
75	GGT TTC GGC ATT AAG AGC AAT GTG ATG GGT ATC GTC ACC TTC TGG CTG 2640 Gly Phe Gly Ile Lys Ser Asn Val Met Gly Ile Val Thr Phe Trp Leu 865 870 875 880	865		870				
80	GAG AAA ATC ACA ATC GGT AGT GAT AAT CCT TTT ACA TTG GCA AAC TAC 2688 Glu Lys Ile Thr Ile Gly Ser Asp Asn Pro Phe Thr Leu Ala Asn Tyr 885 890 895	885		890				

	TGG GAT GAT ATT CAA ACC CTG TTT AGC CAT GAC AAT GCC ACG TTA GAG	2786
	Trp His Asp Ile Gln Thr Leu Phe Ser His Asp Asn Ala Thr Leu Glu	
	900 905 910	
5	TCC TTA CAA ACC GAC ACT TCT CTG GTA ATT GCT ACT CAG CAA CTT AGC	2784
	Ser Leu Gln Thr Asp Thr Ser Leu Val Ile Ala Thr Gln Gln Leu Ser	
	915 920 925	
10	CAG CTA GTG TTA ATT GTG AAA TGG CTG AGC CTG ACC GAG CAG GAT CTG	2832
	Gln Leu Val Leu Ile Val Lys Trp Leu Ser Leu Thr Glu Gln Asp Leu	
	930 935 940	
15	CAA TTA CTG ACA ACC TAT CCC GAA CGT TTA ATC AAC GGC ATC ACG AAT	2880
	Gln Leu Leu Thr Thr Tyr Pro Glu Arg Leu Ile Asn Gly Ile Thr Asn	
	945 950 955 960	
20	GTT CCT GTA CCC AAT CCG GAG CTA TTA CTC ACG CTA TCA CGT TTT AAG	2928
	Val Pro Val Pro Asn Pro Glu Leu Leu Thr Thr Leu Ser Arg Phe Lys	
	965 970 975	
	CAG TGG GAA ACT CAA GTC ACC GTT TCC CGT GAT GAA GCG ATG CGC TGT	2976
	Gln Trp Glu Thr Gln Val Thr Val Ser Arg Asp Glu Ala Met Arg Cys	
	980 985 990	
25	TTC GAT CAA TTA AAT GCC AAT GAT ATG ACG ACT GAA AAT GCA GGT TCA	3024
	Phe Asp Gln Leu Asn Ala Asn Asp Met Thr Thr Glu Asn Ala Gly Ser	
	995 1000 1005	
30	CTG ATC GCC ACA TTG TAT GAG ATG GAT AAA GGT ACG GGA GCG CAA GTT	3072
	Leu Ile Ala Thr Leu Tyr Glu Met Asp Lys Gly Thr Gly Ala Gln Val	
	1010 1015 1020	
35	AAT ACC TTG CTA TTA GGT GAA AAT AAC TGG CCG AAA AGT TTT ACC TCT	3120
	Asn Thr Leu Leu Leu Gly Glu Asn Asn Trp Pro Lys Ser Phe Thr Ser	
	1025 1030 1035 1040	
40	CTC TGG CAA CTT CTG ACC TGG TTA CGC GTC GGG CAA AGA CTG AAT GTC	3168
	Leu Trp Gln Leu Leu Thr Trp Leu Arg Val Gly Gln Arg Leu Asn Val	
	1045 1050 1055	
	GGT AGT ACC ACT CTG GGC AAT CTG TTG TCC ATG ATG CAA GCA GAC CCT	3216
	Gly Ser Thr Thr Leu Gly Asn Leu Leu Ser Met Met Gln Ala Asp Pro	
	1060 1065 1070	
45	GCT GCC GAG AGT AGC GCT TTA TTG GCA TCA GTA GCC CAA AAC TTA AGT	3264
	Ala Ala Glu Ser Ser Ala Leu Leu Ala Ser Val Ala Gln Asn Leu Ser	
	1075 1080 1085	
50	GCC GCA ATC AGC AAT CGT CAG TAA	3285
	Ala Ala Ile Ser Asn Arg Gln ...	
	1090 1095	

(2) INFORMATION FOR SEQ ID NO:34:

55 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1095 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

60 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Features	From	To	Description
65	254	267	SEQ ID NO:15
	254	492	TcaaAii peptide

5 Met Val Thr Val Met Gln Asn Lys Ile Ser Phe Leu Ser Gly Thr Ser  
 1 5 10 15  
 Glu Gln Pro Leu Leu Asp Ala Gly Tyr Gln Asn Val Phe Asp Ile Ala  
 20 25 30  
 10 Ser Ile Ser Arg Ala Thr Phe Val Gln Ser Val Pro Thr Leu Pro Val  
 35 40 45  
 Lys Glu Ala His Thr Val Tyr Arg Gln Ala Arg Gln Arg Ala Glu Asn  
 50 55 60  
 15 Leu Lys Ser Leu Tyr Arg Ala Trp Gln Leu Arg Gln Glu Pro Val Ile  
 65 70 75 80  
 Lys Gly Leu Ala Lys Leu Asn Leu Gln Ser Asn Val Ser Val Leu Gln  
 85 90 95  
 20 Asp Ala Leu Val Glu Asn Ile Gly Gly Asp Gly Asp Phe Ser Asp Leu  
 100 105 110  
 Met Asn Arg Ala Ser Gln Tyr Ala Asp Ala Ala Ser Ile Gln Ser Leu  
 115 120 125  
 Phe Ser Pro Gly Arg Tyr Ala Ser Ala Leu Tyr Arg Val Ala Lys Asp  
 130 135 140  
 30 Leu His Lys Ser Asp Ser Ser Leu His Ile Asp Asn Arg Arg Ala Asp  
 145 150 155 160  
 Leu Lys Asp Leu Ile Leu Ser Glu Thr Thr Met Asn Lys Glu Val Thr  
 165 170 175  
 35 Ser Leu Asp Ile Leu Leu Asp Val Leu Gln Lys Gly Gly Lys Asp Ile  
 180 185 190  
 Thr Glu Leu Ser Gly Ala Phe Phe Pro Met Thr Leu Pro Tyr Asp Asp  
 195 200 205  
 His Leu Ser Gln Ile Asp Ser Ala Leu Ser Ala Gln Ala Arg Thr Leu  
 210 215 220  
 45 Asn Gly Val Trp Asn Thr Leu Thr Asp Thr Thr Ala Gln Ala Val Ser  
 225 230 235 240  
 Glu Gln Thr Ser Asn Thr Asn Thr Arg Lys Leu Phe Ala Ala Gln Asp  
 245 250 255  
 50 Gly Asn Gln Asp Thr Phe Phe Ser Gly Asn Thr Phe Tyr Phe Lys Ala  
 260 265 270  
 Val Gly Phe Ser Gly Gln Pro Met Val Tyr Leu Ser Gln Tyr Thr Ser  
 275 280 285  
 Gly Asn Gly Ile Val Gly Ala Gln Leu Ile Ala Gly Asn Pro Asp Gln  
 290 295 300  
 60 Ala Ala Ala Ala Ile Val Ala Pro Leu Lys Leu Thr Trp Ser Met Ala  
 305 310 315 320  
 Lys Gln Cys Tyr Tyr Leu Val Ala Pro Asp Gly Thr Thr Met Gly Asp  
 325 330 335  
 65 Gly Asn Val Leu Thr Gly Cys Phe Leu Arg Gly Asn Ser Pro Thr Asn

	340	345	350
	Pro Asp Lys Asp Gly Ile Phe Ala Gln Val Ala Asn Lys Ser Gly Ser		
	355	360	365
5	Thr Gln Pro Leu Pro Ser Phe His Leu Pro Val Thr Leu Glu His Ser		
	370	375	380
10	Glu Asn Lys Asp Gln Tyr Tyr Leu Lys Thr Glu Gln Gly Tyr Ile Thr		
	385	390	395
	Val Asp Ser Ser Gly Gln Ser Asn Trp Lys Asn Ala Leu Val Ile Asn		
	405	410	415
15	Gly Thr Lys Asp Lys Gly Leu Leu Leu Thr Phe Cys Ser Asp Ser Ser		
	420	425	430
	Gly Thr Pro Thr Asn Pro Asp Asp Val Ile Pro Pro Ala Ile Asn Asp		
	435	440	445
20	Ile Pro Ser Pro Pro Ala Arg Glu Thr Leu Ser Leu Thr Pro Val Ser		
	450	455	460
25	Tyr Gln Leu Met Thr Asn Pro Ala Pro Thr Glu Asp Asp Ile Thr Asn		
	465	470	475
	His Tyr Gly Phe Asn Gly Ala Ser Leu Arg Ala Ser Pro Leu Ser Thr		
	485	490	495
30	Ser Glu Leu Thr Ser Lys Leu Asn Ser Ile Asp Thr Phe Cys Glu Lys		
	500	505	510
	Thr Arg Leu Ser Phe Asn Gln Leu Met Asp Leu Thr Ala Gln Gln Ser		
	515	520	525
35	Tyr Ser Gln Ser Ser Ile Asp Ala Lys Ala Ala Ser Arg Tyr Val Arg		
	530	535	540
40	Phe Gly Glu Thr Thr Pro Thr Arg Val Asn Val Tyr Gly Ala Ala Tyr		
	545	550	555
	Leu Asn Ser Thr Leu Ala Asp Ala Ala Asp Gly Gln Tyr Leu Trp Ile		
	565	570	575
45	Gln Thr Asp Gly Lys Ser Leu Asn Phe Thr Asp Asp Thr Val Val Ala		
	580	585	590
	Leu Ala Gly Arg Ala Glu Lys Leu Val Arg Leu Ser Ser Gln Thr Gly		
	595	600	605
50	Leu Ser Phe Glu Glu Leu Asp Trp Leu Ile Ala Asn Ala Ser Arg Ser		
	610	615	620
55	Val Pro Asp His His Asp Lys Ile Val Leu Asp Lys Pro Val Leu Glu		
	625	630	635
	Ala Leu Ala Glu Tyr Val Ser Leu Lys Gln Arg Tyr Gly Leu Asp Ala		
	645	650	655
60	Asn Thr Phe Ala Thr Phe Ile Ser Ala Val Asn Pro Tyr Thr Pro Asp		
	660	665	670
	Gln Thr Pro Ser Phe Tyr Glu Thr Ala Phe Arg Ser Ala Asp Gly Asn		
	675	680	685
65	His Val Ile Ala Leu Gly Thr Glu Val Lys Tyr Ala Glu Asn Glu Gln		

	690	695	700	
5	Asp Glu Leu Ala Ala Ile Cys Cys Lys Ala Leu Gly Val Thr Ser Asp 705 710 715 720			
	Glu Leu Leu Arg Ile Gly Arg Tyr Cys Phe Gly Asn Ala Gly Ser Phe 725 730 735			
10	Thr Leu Asp Glu Tyr Thr Ala Ser Gln Leu Tyr Arg Phe Gly Ala Ile 740 745 750			
	Pro Arg Leu Phe Gly Leu Thr Phe Ala Gln Ala Glu Ile Leu Trp Arg 755 760 765			
15	Leu Met Glu Gly Gly Lys Asp Ile Leu Leu Gln Gln Leu Gly Gln Ala 770 775 780			
	Lys Ser Leu Gln Pro Leu Ala Ile Leu Arg Arg Thr Glu Gln Val Leu 785 790 795 800			
20	Asp Trp Met Ser Ser Val Asn Leu Ser Leu Thr Tyr Leu Gln Gly Met 805 810 815			
	Val Ser Thr Gln Trp Ser Gly Thr Ala Thr Ala Glu Met Phe Asn Phe 820 825 830			
	Leu Glu Asn Val Cys Asp Ser Val Asn Ser Gln Ala Ala Thr Lys Glu 835 840 845			
30	Thr Met Asp Ser Ala Leu Gln Gln Lys Val Leu Arg Ala Leu Ser Ala 850 855 860			
	Gly Phe Gly Ile Lys Ser Asn Val Met Gly Ile Val Thr Phe Trp Leu 865 870 875 880			
35	Glu Lys Ile Thr Ile Gly Ser Asp Asn Pro Phe Thr Leu Ala Asn Tyr 885 890 895			
	Trp His Asp Ile Gln Thr Leu Phe Ser His Asp Asn Ala Thr Leu Glu 900 905 910			
	Ser Leu Gln Thr Asp Thr Ser Leu Val Ile Ala Thr Gln Gln Leu Ser 915 920 925			
45	Gln Leu Val Leu Ile Val Lys Trp Leu Ser Leu Thr Glu Gln Asp Leu 930 935 940			
	Gln Leu Leu Thr Thr Tyr Pro Glu Arg Leu Ile Asn Gly Ile Thr Asn 945 950 955 960			
50	Val Pro Val Pro Asn Pro Glu Leu Leu Leu Thr Leu Ser Arg Phe Lys 965 970 975			
	Gln Trp Glu Thr Gln Val Thr Val Ser Arg Asp Glu Ala Met Arg Cys 980 985 990			
	Phe Asp Gln Leu Asn Ala Asn Asp Met Thr Thr Glu Asn Ala Gly Ser 995 1000 1005			
60	Leu Ile Ala Thr Leu Tyr Glu Met Asp Lys Gly Thr Gly Ala Gln Val 1010 1015 1020			
	Asn Thr Leu Leu Leu Gly Glu Asn Asn Trp Pro Lys Ser Phe Thr Ser 1025 1030 1035 1040			
65	Leu Trp Gln Leu Leu Thr Trp Leu Arg Val Gly Gln Arg Leu Asn Val			

1045 1050 1055

Gly Ser Thr Thr Leu Gly Asn Leu Leu Ser Met Met Gln Ala Asp Pro  
1060 1065 1070

5 Ala Ala Glu Ser Ser Ala Leu Leu Ala Ser Val Ala Gln Asn Leu Ser  
1075 1080 1085

Ala Ala Ile Ser Asn Arg Gln ...  
1090 1095

10

(2) INFORMATION FOR SEQ ID NO:35  
(i) SEQUENCE CHARACTERISTICS:  
15 (A) LENGTH: 603 amino acids  
(B) TYPE: amino acid  
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein  
20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

25 Pro Leu Ser Thr Ser Glu Leu Thr Ser Lys Leu Asn Ser Ile Asp Thr  
1 5 10 15

Phe Cys Glu Lys Thr Arg Leu Ser Phe Asn Gln Leu Met Asp Leu Thr  
20 25 30

30 Ala Gln Gln Ser Tyr Ser Gln Ser Ser Ile Asp Ala Lys Ala Ala Ser  
35 40 45

Arg Tyr Val Arg Phe Gly Glu Thr Thr Pro Thr Arg Val Asn Val Tyr  
50 55 60

35 Gly Ala Ala Tyr Leu Asn Ser Thr Leu Ala Asp Ala Ala Asp Gly Gln  
65 70 75 80

Tyr Leu Trp Ile Gln Thr Asp Gly Lys Ser Leu Asn Phe Thr Asp Asp  
40 85 90 95

Thr Val Val Ala Leu Ala Gly Arg Ala Glu Lys Leu Val Arg Leu Ser  
100 105 110

45 Ser Gln Thr Gly Leu Ser Phe Glu Glu Leu Asp Trp Leu Ile Ala Asn  
115 120 125

Ala Ser Arg Ser Val Pro Asp His His Asp Lys Ile Val Leu Asp Lys  
130 135 140

50 Pro Val Leu Glu Ala Leu Ala Glu Tyr Val Ser Leu Lys Gln Arg Tyr  
145 150 155 160

Gly Leu Asp Ala Asn Thr Phe Ala Thr Phe Ile Ser Ala Val Asn Pro  
55 165 170 175

Tyr Thr Pro Asp Gln Thr Pro Ser Phe Tyr Glu Thr Ala Phe Arg Ser  
180 185 190

60 Ala Asp Gly Asn His Val Ile Ala Leu Gly Thr Glu Val Lys Tyr Ala  
195 200 205

Glu Asn Glu Gln Asp Glu Leu Ala Ala Ile Cys Cys Lys Ala Leu Gly  
210 215 220

65



	Val	Thr	Ser	Asp	Glu	Leu	Leu	Arg	Ile	Gly	Arg	Tyr	Cys	Phe	Gly	Asn	225	230	235	240
5	Ala	Gly	Arg	Phe	Thr	Leu	Asp	Glu	Tyr	Thr	Ala	Ser	Gln	Leu	Tyr	Arg	245	250	255	
	Phe	Gly	Ala	Ile	Pro	Arg	Leu	Phe	Gly	Leu	Thr	Phe	Ala	Gln	Ala	Glu	260	265	270	
10	Ile	Leu	Trp	Arg	Leu	Met	Glu	Gly	Gly	Lys	Asp	Ile	Leu	Leu	Gln	Gln	275	280	285	
	Kxx	Gly	Gln	Ala	Lys	Ser	Leu	Gln	Pro	Leu	Ala	Ile	Leu	Arg	Arg	Thr	290	295	300	
15	Glu	Gln	Val	Leu	Asp	Trp	Met	Ser	Pro	Val	Asn	Leu	Ser	Leu	Thr	Tyr	305	310	315	320
	Leu	Gln	Gly	Met	Val	Ser	Thr	Gln	Trp	Ser	Gly	Thr	Ala	Thr	Ala	Glu	325	330	335	
20	Met	Phe	Asn	Phe	Leu	Glu	Asn	Val	Cys	Asp	Ser	Val	Asn	Ser	Gln	Ala	340	345	350	
	Kxx	Thr	Lys	Glu	Thr	Met	Asp	Ser	Ala	Leu	Gln	Gln	Lys	Val	Leu	Arg	355	360	365	
	Ala	Leu	Ser	Ala	Gly	Phe	Gly	Ile	Lys	Ser	Asn	Val	Met	Gly	Ile	Val	370	375	380	
30	Thr	Phe	Trp	Leu	Glu	Lys	Ile	Thr	Ile	Gly	Arg	Asp	Asn	Pro	Phe	Thr	385	390	395	400
	Leu	Ala	Asn	Tyr	Trp	His	Asp	Ile	Gln	Thr	Leu	Phe	Ser	His	Asp	Asn	405	410	415	
35	Ala	Thr	Leu	Glu	Ser	Leu	Gln	Thr	Asp	Thr	Ser	Leu	Val	Ile	Ala	Thr	420	425	430	
	Gln	Gln	Leu	Ser	Gln	Leu	Val	Leu	Ile	Val	Lys	Trp	Val	Ser	Leu	Thr	435	440	445	
40	Glu	Gln	Asp	Leu	Gln	Leu	Leu	Thr	Thr	Tyr	Pro	Glu	Arg	Leu	Ile	Asn	450	455	460	
45	Gly	Ile	Thr	Asn	Val	Pro	Val	Pro	Asn	Pro	Glu	Leu	Leu	Leu	Thr	Leu	465	470	475	480
	Ser	Arg	Phe	Lys	Gln	Trp	Glu	Thr	Gln	Val	Thr	Val	Ser	Arg	Asp	Glu	485	490	495	
50	Ala	Met	Arg	Cys	Phe	Asp	Gln	Leu	Asn	Ala	Asn	Asp	Met	Thr	Thr	Glu	500	505	510	
	Asn	Ala	Gly	Ser	Leu	Ile	Ala	Thr	Leu	Tyr	Glu	Met	Asp	Lys	Gly	Thr	515	520	525	
	Gly	Ala	Gln	Val	Asn	Thr	Leu	Leu	Leu	Gly	Glu	Asn	Asn	Trp	Pro	Lys	530	535	540	
60	Ser	Phe	Thr	Ser	Leu	Trp	Gln	Leu	Leu	Thr	Trp	Leu	Arg	Val	Gly	Gln	545	550	555	560
	Arg	Leu	Asn	Val	Gly	Ser	Thr	Thr	Leu	Gly	Asn	Leu	Leu	Ser	Met	Met	565	570	575	

Gln Ala Asp Pro Ala Ala Glu Ser Ser Ala Leu Leu Ala Ser Val Ala  
580 585 590

5 Gln Asn Leu Ser Ala Ala Ile Ser Asn Arg Gln \*  
595 600

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 2557 base pairs  
(B) TYPE: nucleic acid  
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

	GAATTCGGCT	TGCGTTTAAT	ATTGATGATG	TCTCGCTCTT	CCGCCTGCTT	AAAATTACCG	60
20	ACCATGATAA	TAAAGATGGA	AAAATTAAAA	ATAACCTAAA	GAATCTTTCC	AATTTATATA	120
	TTGGAATAAT	ACTGGCAGAT	ATTCATCAAT	TAACCATTGA	TGAACTGGAT	TTATTACTGA	180
	TTGCCGTAGG	TGAAGGAAAA	ACTAATTTAT	CCGCTATCAG	TGATAAGCAA	TTGGCTACCC	240
	TGATCAGAAA	ACTCAATACT	ATTACCAGCT	GGCTACATAC	ACAGAAGTGG	AGTGTATTCC	300
	AGCTATTTAT	CATGACCTCC	ACCAGCTATA	ACAAAACGCT	AACGCCTGAA	ATTAAGAATT	360
25	TGCTGGATAC	CGTCTACCAC	GGTTTACAAG	GTTTTGATAA	AGACAAAGCA	GATTTGCTAC	420
	ATGTCATGGC	GCCCTATATT	GCGGCCACCT	TGCAATTATC	ATCGGAAAAT	GTCGCCCCACT	480
	CGGTACTCCT	TTGGGCAGAT	AAGTTACAGC	CCGGCGACGG	CGCAATGACA	GCAGAGGGAN	540
	TCTGGGACTG	GTTGAATACT	AAGTATACGC	CGGGTTTCATC	GGAAGCCGTA	GAAACGCAGG	600
	AACATATCGT	TCAGTATTGT	CAGGCTCTGG	CACAATTGGA	AATGGTTTAC	CATTCCACCG	660
30	GCATCAACGA	AAACGCCTTC	CGTCTATTTG	TGACAAAACC	AGAGATGTTT	GGCGCTGCAA	720
	CTGGAGCAGC	GCCCCGCGCAT	GATGCCCTTT	CACTGATTAT	GCTGACACGT	TTTGCGGATT	780
	GGGTGAACGC	ACTAGGCGAA	AAAGCGTCCT	CGGTGCTAGC	GGCATTTGAA	GCTAACTCGT	840
	TAACGGCAGA	ACAACCTGGCT	GATGCCATGA	ATCTTGATGC	TAATTTGCTG	TTGCAAGCCA	900
	GTATTCAAGC	ACAAAATCAT	CAACATCTTC	CCCCAGTAAC	TCCAGAAAAT	GCGTTCTCCT	960
35	GTTGGACATC	TATCAATACT	ATCCTGCAAT	GGGTTAATGT	CGCACAAACA	TTGAAATGTC	1020
	GCCCCACAGG	GCGTTTCCGC	TTTGGTCGGG	CTGGATTATA	TTCAATCAAT	GAAAGAGACA	1080
	CCGACCTATG	CCCAGTGGA	AAACGCGGCA	GGCGTATTAA	CCGCCGGGTT	GAATTCAACA	1140
	ACAGGCTAAT	ACATTACAAC	GCTTTTCTGG	ATGAATCTCG	CAGTGCCGCA	TTAAGCACCT	1200
	ACTATATCCG	TCAAGTCGCC	AAGGCAGCGG	CGGCTATTAA	AAGCCGTGAT	GACTTGTATC	1260
40	AATACTTACT	GATTGATAAT	CAGGTTTCTG	CGGCAATAAA	AACCACCCGG	ATCGCCGAAG	1320
	CCATTGCCAG	TATTCAACTG	TACGTCAACC	GGGCATTGGA	AAATGTGGAA	GAAAATGCCA	1380
	ATTCGGGGGT	TATCAGCCGC	CAATTCTTTA	TCGACTGGGA	CAAATACAAT	AAACGCTACA	1440
	GCACTTGGGC	GGGTGTTTCT	CAATTAGTTT	ACTACCCGGA	AAACTATATT	GATCCGACCA	1500
	TGCGTATCGG	ACAAACCAAA	ATGATGGACG	CATTACTGCA	ATCCGTCAGC	CAAAGCCAAT	1560
45	TAAACGCCGA	TACCGTCGAA	GATGCCTTTA	TGTCTTATCT	GACATCGTTT	GAACAAGTGG	1620
	CTAATCTTAA	AGTTATTAGC	GCATATCAGC	ATAATATTAA	TAACGATCAA	GGGCTGACCT	1680
	ATTTTATCGG	ACTCAGTGAA	ACTGATGCCG	GTGAATATTA	TTGGCGCAGT	GTCGATCACA	1740
	GTAAATTCAA	CGACGGTAAA	TTCGCGGCTA	ATGCCTGGAG	TGAATGGCAT	AAAATTGATT	1800
	GTCCAATTAA	CCCTTATAAA	AGCACTATCC	GTCCAGTGAT	ATATAAATCC	CGCCTGTATC	1860

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TGCTCTGGTT GGAACAAAAG GAGATCACCA AACAGACAGG AAATAGTAA GATGGCTATC 1920
AAACTGAAAC GGATTATCGT TATGAACTAA AATTGGCGCA TATCCGCTAT GATGGCACTT 1980
GGAATACGCC AATCACCTTT GATGTCAATA AAAAAATATC CGAGCTAAAA CTGGAAAAAA 2040
ATAGAGCGCC CGGACTCTAT TGTGCCGGTT ATCAAGGTGA AGATACGTTG CTGGTGATGT 2100
5 TTTATAACCA ACAAGACACA CTAGATAGTT ATAAAAACGC TTCAATGCAA GGACTATATA 2160
TCTTTGCTGA TATGGCATCC AAAGATATGA CCCCAGAACA GAGCAATGTT TATCGGGATA 2220
ATAGCTATCA ACAATTTGAT ACCAATAATG TCAGAAGAGT GAATAACCGC TATGCAGAGG 2280
ATTATGAGAT TCCTTCTTCG GTAAGTAGCC GTAAAGACTA TGGTTGGGGA GATTATTACC 2340
TCAGCATGGT ATATAACGGA GATATTCCAA CTATCAATTA CAAAGCCGCA TCAAGTGATT 2400
10 TAAAAATTTA TATTTACCA AAATTAAGAA TTATTCATAA TGGATATGAA GGACAGAAGC 2460
GCAATCAATG CAATTTGATG AATAAATATG GCAAAC TAGG TGATAAATTT ATTGTGTATA 2520
CCAGCCTGGG CGTTAATCCG AATAATAAGC CGAATTC 2557

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15 (2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 845 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: protein (partial)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

25 Ala Phe Asn Ile Asp Asp Val Ser Leu Phe Arg Leu Leu Lys Ile Thr  
1 5 10 15

30 Asp His Asp Asn Lys Asp Gly Lys Ile Lys Asn Asn Leu Lys Asn Leu  
20 25 30

Ser Asn Leu Tyr Ile Gly Lys Leu Leu Ala Asp Ile His Gln Leu Thr  
35 40 45

35 Ile Asp Glu Leu Asp Leu Leu Leu Ile Ala Val Gly Glu Gly Lys Thr  
50 55 60

Asn Leu Ser Ala Ile Ser Asp Lys Gln Leu Ala Thr Leu Ile Arg Lys  
40 65 70 75 80

Leu Asn Thr Ile Thr Ser Trp Leu His Thr Gln Lys Trp Ser Val Phe  
85 90 95

45 Gln Leu Phe Ile Met Thr Ser Thr Ser Tyr Asn Lys Thr Leu Thr Pro  
100 105 110

Glu Ile Lys Asn Leu Leu Asp Thr Val Tyr His Gly Leu Gln Gly Phe  
115 120 125

50 Asp Lys Asp Lys Ala Asp Leu Leu His Val Met Ala Pro Tyr Ile Ala  
130 135 140

Ala Thr Leu Gln Leu Ser Ser Glu Asn Val Ala His Ser Val Leu Leu  
145 150 155 160

55 Trp Ala Asp Lys Leu Gln Pro Gly Asp Gly Ala Met Thr Ala Glu Gly  
165 170 175

Phe Trp Asp Trp Leu Asn Thr Lys Tyr Thr Pro Gly Ser Ser Glu Ala

	180							185							190									
5	Val	Glu	Thr	Gln	Glu	His	Ile	Val	Gln	Tyr	Cys	Gln	Ala	Leu	Ala	Gln								
			195						200				205											
	Leu	Glu	Met	Val	Tyr	His	Ser	Thr	Gly	Ile	Asn	Glu	Asn	Ala	Phe	Arg								
			210				215						220											
10	Leu	Phe	Val	Thr	Lys	Pro	Glu	Met	Phe	Gly	Ala	Ala	Thr	Gly	Ala	Ala								
						230					235					240								
	Pro	Ala	His	Asp	Ala	Leu	Ser	Leu	Ile	Met	Leu	Thr	Arg	Phe	Ala	Asp								
					245					250					255									
15	Trp	Val	Asn	Ala	Leu	Gly	Glu	Lys	Ala	Ser	Ser	Val	Leu	Ala	Ala	Phe								
				260					265						270									
	Glu	Ala	Asn	Ser	Leu	Thr	Ala	Glu	Gln	Leu	Ala	Asp	Ala	Met	Asn	Leu								
			275						280				285											
20	Asp	Ala	Asn	Leu	Leu	Leu	Gln	Ala	Ser	Ile	Gln	Ala	Gln	Asn	His	Gln								
			290				295					300												
25	His	Leu	Pro	Pro	Val	Thr	Pro	Glu	Asn	Ala	Phe	Ser	Cys	Trp	Thr	Ser								
						310					315					320								
	Ile	Asn	Thr	Ile	Leu	Gln	Trp	Val	Asn	Val	Ala	Gln	Gln	Leu	Lys	Cys								
					325				330						335									
30	Arg	Pro	Thr	Gly	Arg	Phe	Arg	Phe	Gly	Arg	Ala	Gly	Leu	Tyr	Ser	Ile								
				340					345					350										
	Asn	Glu	Arg	Asp	Thr	Asp	Leu	Cys	Pro	Val	Gly	Lys	Arg	Gly	Arg	Arg								
			355						360				365											
35	Ile	Asn	Arg	Arg	Val	Glu	Phe	Asn	Asn	Arg	Leu	Ile	His	Tyr	Asn	Ala								
			370				375					380												
40	Phe	Leu	Asp	Glu	Ser	Arg	Ser	Ala	Ala	Leu	Ser	Thr	Tyr	Tyr	Ile	Arg								
						390					395				400									
	Gln	Val	Ala	Lys	Ala	Ala	Ala	Ala	Ile	Lys	Ser	Arg	Asp	Asp	Leu	Tyr								
					405				410						415									
45	Gln	Tyr	Leu	Leu	Ile	Asp	Asn	Gln	Val	Ser	Ala	Ala	Ile	Lys	Thr	Thr								
				420					425</															

	530	535	540
	Tyr His Asp Asn Ile Asn Asn Asp Gln Gly Leu Thr Tyr Phe Ile Gly		
	545	550	555 560
5	Leu Ser Glu Thr Asp Ala Gly Glu Tyr Tyr Trp Arg Ser Val Asp His		
		565	570 575
10	Ser Lys Phe Asn Asp Gly Lys Phe Ala Ala Asn Ala Trp Ser Glu Trp		
		580	585 590
	His Lys Ile Asp Cys Pro Ile Asn Pro Tyr Lys Ser Thr Ile Arg Pro		
		595	600 605
15	Val Ile Tyr Lys Ser Arg Leu Tyr Leu Leu Trp Leu Glu Gln Lys Glu		
		610	615 620
	Ile Thr Lys Gln Thr Gly Asn Ser Lys Asp Gly Tyr Gln Thr Glu Thr		
		625	630 635 640
20	Asp Tyr Arg Tyr Glu Leu Lys Leu Ala His Ile Arg Tyr Asp Gly Thr		
		645	650 655
25	Trp Asn Thr Pro Ile Thr Phe Asp Val Asn Lys Lys Ile Ser Glu Leu		
		660	665 670
	Lys Leu Glu Lys Asn Arg Ala Pro Gly Leu Tyr Cys Ala Gly Tyr Gln		
		675	680 685
30	Gly Glu Asp Thr Leu Leu Val Met Phe Tyr Asn Gln Gln Asp Thr Leu		
		690	695 700
	Asp Ser Tyr Lys Asn Ala Ser Met Gln Gly Leu Tyr Ile Phe Ala Asp		
		705	710 715 720
35	Met Ala Ser Lys Asp Met Thr Pro Glu Gln Ser Asn Val Tyr Arg Asp		
		725	730 735
40	Asn Ser Tyr Gln Gln Phe Asp Thr Asn Asn Val Arg Arg Val Asn Asn		
		740	745 750
	Arg Tyr Ala Glu Asp Tyr Glu Ile Pro Ser Ser Val Ser Ser Arg Lys		
		755	760 765
45	Asp Tyr Gly Trp Gly Asp Tyr Tyr Leu Ser Met Val Tyr Asn Gly Asp		
		770	775 780
	Ile Pro Thr Ile Asn Tyr Lys Ala Ala Ser Ser Asp Leu Lys Ile Tyr		
		785	790 795 800
50	Ile Ser Pro Lys Leu Arg Ile Ile His Asn Gly Tyr Glu Gly Gln Lys		
		805	810 815
55	Arg Asn Gln Cys Asn Leu Met Asn Lys Tyr Gly Lys Leu Gly Asp Lys		
		820	825 830
	Phe Ile Val Tyr Thr Ser Leu Gly Val Asn Pro Asn Asn		
		835	840 845

60

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

65

- (A) LENGTH: 16 amino acids  
 (B) TYPE: amino acid

(C) STRANDNESS: single  
(D) TOPOLCGT: linear

(ii) MOLECULAR TYPE: protein

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Arg Tyr Tyr Asn Leu Ser Asp Glu Glu Leu Ser Gln Phe Ile Gly  
1 5 10 15  
Lys

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 amino acids  
(B) TYPE: amino acid  
(C) STRANDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: protein

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Gly Thr Ala Thr Asp Val Ser Gly Pro Val Glu Ile Asn Thr Ala  
1 5 10 15  
Ile Ser Pro Ala Lys  
20

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 11 amino acids  
(B) TYPE: amino acid  
(C) STRANDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: protein

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Ala Asn Ser Leu Tyr Ala Leu Phe Leu Pro Gln  
1 5 10

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 14 amino acids

(B) TYPE: amino acid  
 (C) STRANDNESS: single  
 (D) TOPOLOGY: linear

5 (ii) MOLECULAR TYPE: protein

(v) FRAGMENT TYPE: N-terminal

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Leu Arg Ser Ala Asn Thr Leu Thr Asp Leu Phe Leu Pro Gln  
 1 5 10

15 (2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 amino acids  
 20 (B) TYPE: amino acid  
 (C) STRANDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: protein

25 (v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

30 Arg Ala Leu Glu Val Glu Arg Thr Val Ser Leu Ala Glu Val Tyr  
 1 5 10 15

Ala Gly Leu Glu

35

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 amino acids  
 40 (B) TYPE: amino acid  
 (C) STRANDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: protein

45

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

50

Ile Arg Glu Asp Tyr Pro Ala Ser Leu Gly Lys  
 1 5 10

55 (2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16 amino acids  
 60 (B) TYPE: amino acid  
 (C) STRANDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: protein

(v) FRAGMENT TYPE: N-terminal

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

10 Asp Asp Ser Gly Asp Asp Asp Lys Val Thr Asn Thr Asp Ile His  
1 5 10 15  
Arg

15 (2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13 amino acids

(B) TYPE: amino acid

20 (C) STRANDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: protein

25 (v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

30 Asp Val Xaa Gly Ser Glu Lys Ala Asn Glu Lys Leu Lys  
1 5 10

(2) INFORMATION FOR SEQ ID NO:46:

35 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7551 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

40 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46 (ccdA):

45

ATG AAC GAG TCT GTA AAA GAG ATA CCT GAT GTA TTA AAA AGC CAG TGT 48  
Met Asn Glu Ser Val Lys Glu Ile Pro Asp Val Leu Lys Ser Gln Cys  
1 5 10 15

50

GGT TTT AAT TGT CTG ACA GAT ATT AGC CAC AGC TCT TTT AAT GAA TTT 96  
Gly Phe Asn Cys Leu Thr Asp Ile Ser His Ser Ser Phe Asn Glu Phe  
20 25 30

55

CGC CAG CAA GTA TCT GAG CAC CTC TCC TGG TCC GAA ACA CAC GAC TTA 144  
Arg Gln Gln Val Ser Glu His Leu Ser Trp Ser Glu Thr His Asp Leu  
35 40 45

60

TAT CAT GAT GCA CAA CAG GCA CAA AAG GAT AAT CGC CTG TAT GAA GCG 192  
Tyr His Asp Ala Gln Gln Ala Gln Lys Asp Asn Arg Leu Tyr Glu Ala  
50 55 60

65

CGT ATT CTC AAA CGC GCC AAT CCC CAA TTA CAA AAT GCG GTG CAT CTT 240  
Arg Ile Leu Lys Arg Ala Asn Pro Gln Leu Gln Asn Ala Val His Leu  
65 70 75 80



	GCC ATT CTC GCT CCC AAT GCT GAA CTG ATA GGC TAT AAC AAT CAA TTT	288
	Ala Ile Leu Ala Pro Asn Ala Glu Leu Ile Gly Tyr Asn Asn Gln Phe	
	85 90 95	
5	AGC GGT AGA GCC AGT CAA TAT GTT CCG CCG GGT ACC GTT TCT TCC ATG	336
	Ser Gly Arg Ala Ser Gln Tyr Val Ala Pro Gly Thr Val Ser Ser Met	
	100 105 110	
10	TTC TCC CCC GCC GCT TAT TTG ACT GAA CTT TAT CGT GAA GCA CGC AAT	384
	Phe Ser Pro Ala Ala Tyr Leu Thr Glu Leu Tyr Arg Glu Ala Arg Asn	
	115 120 125	
15	TTA CAC GCA AGT GAC TCC GTT TAT TAT CTG GAT ACC CGC CGC CCA GAT	432
	Leu His Ala Ser Asp Ser Val Tyr Tyr Leu Asp Thr Arg Arg Pro Asp	
	130 135 140	
20	CTC AAA TCA ATG GCG CTC AGT CAG CAA AAT ATG GAT ATA GAA TTA TCC	480
	Leu Lys Ser Met Ala Leu Ser Gln Gln Asn Met Asp Ile Glu Leu Ser	
	145 150 155 160	
	ACA CTC TCT TTG TCC AAT GAG CTG TTA TTG GAA AGC ATT AAA ACT GAA	528
	Thr Leu Ser Leu Ser Asn Glu Leu Leu Leu Glu Ser Ile Lys Thr Glu	
	165 170 175	
25	TCT AAA CTG GAA AAC TAT ACT AAA GTG ATG GAA ATG CTC TCC ACT TTC	576
	Ser Lys Leu Glu Asn Tyr Thr Lys Val Met Glu Met Leu Ser Thr Phe	
	180 185 190	
30	CGT CCT TCC GGC GCA ACG CCT TAT CAT GAT GCT TAT GAA AAT GTG CGT	624
	Arg Pro Ser Gly Ala Thr Pro Tyr His Asp Ala Tyr Glu Asn Val Arg	
	195 200 205	
35	GAA GTT ATC CAG CTA CAA GAT CCT GGA CTT GAG CAA CTC AAT GCA TCA	672
	Glu Val Ile Gln Leu Gln Asp Pro Gly Leu Glu Gln Leu Asn Ala Ser	
	210 215 220	
40	CCG GCA ATT GCC GGG TTG ATG CAT CAA GCC TCC CTA TTG GGT ATT AAC	720
	Pro Ala Ile Ala Gly Leu Met His Gln Ala Ser Leu Leu Gly Ile Asn	
	225 230 235 240	
	GCT TCA ATC TCG CCT GAG CTA TTT AAT ATT CTG ACG GAG GAG ATT ACC	768
	Ala Ser Ile Ser Pro Glu Leu Phe Asn Ile Leu Thr Glu Glu Ile Thr	
	245 250 255	
45	GAA GGT AAT GCT GAG GAA CTT TAT AAG AAA AAT TTT GGT AAT ATC GAA	816
	Glu Gly Asn Ala Glu Glu Leu Tyr Lys Lys Asn Phe Gly Asn Ile Glu	
	260 265 270	
50	CCG GCC TCA TTG GCT ATG CCG GAA TAC CTT AAA CGT TAT TAT AAT TTA	864
	Pro Ala Ser Leu Ala Met Pro Glu Tyr Leu Lys Arg Tyr Tyr Asn Leu	
	275 280 285	
55	AGC GAT GAA GAA CTT AGT CAG TTT ATT GGT AAA GCC AGC AAT TTT GGT	912
	Ser Asp Glu Glu Leu Ser Gln Phe Ile Gly Lys Ala Ser Asn Phe Gly	
	290 295 300	
60	CAA CAG GAA TAT AGT AAT AAC CAA CTT ATT ACT CCG GTA GTC AAC AGC	960
	Gln Gln Glu Tyr Ser Asn Asn Gln Leu Ile Thr Pro Val Val Asn Ser	
	305 310 315 320	
	AGT GAT GGC ACG GTT AAG GTA TAT CGG ATC ACC CGC GAA TAT ACA ACC	1008
	Ser Asp Gly Thr Val Lys Val Tyr Arg Ile Thr Arg Glu Tyr Thr Thr	
	325 330 335	
65	AAT GCT TAT CAA ATG GAT GTG GAG CTA TTT CCC TTC GGT GGT GAG AAT	1056
	Asn Ala Tyr Gln Met Asp Val Glu Leu Phe Pro Phe Gly Gly Glu Asn	
	340 345 350	
70	TAT CGG TTA GAT TAT AAA TTC AAA AAT TTT TAT AAT GCC TCT TAT TTA	1104
	Tyr Arg Leu Asp Tyr Lys Phe Lys Asn Phe Tyr Asn Ala Ser Tyr Leu	
	355 360 365	

5	TCC	ATC	AAG	TTA	AAT	GAT	AAA	AGA	GAA	CTT	GTT	CGA	ACT	GAA	GGC	GCT	1152
	Ser	Ile	Lys	Leu	Asn	Asp	Lys	Arg	Glu	Leu	Val	Arg	Thr	Glu	Gly	Ala	
	370						375					380					
10	CCT	CAA	GTC	AAT	ATA	GAA	TAC	TCC	GCA	AAT	ATC	ACA	TTA	AAT	ACC	GCT	1200
	Pro	Gln	Val	Asn	Ile	Glu	Tyr	Ser	Ala	Asn	Ile	Thr	Leu	Asn	Thr	Ala	
	385					390					395					400	
15	GAT	ATC	AGT	CAA	CCT	TTT	GAA	ATT	GGC	CTG	ACA	CGA	GTA	CTT	CCT	TCC	1248
	Asp	Ile	Ser	Gln	Pro	Phe	Glu	Ile	Gly	Leu	Thr	Arg	Val	Leu	Pro	Ser	
					405					410					415		
20	GGT	TCT	TGG	GCA	TAT	GCC	GCC	GCA	AAA	TTT	ACC	GTT	GAA	GAG	TAT	AAC	1296
	Gly	Ser	Trp	Ala	Tyr	Ala	Ala	Ala	Lys	Phe	Thr	Val	Glu	Glu	Tyr	Asn	
				420				425					430				
25	CAA	TAC	TCT	TTT	CTG	CTA	AAA	CTT	AAC	AAG	GCT	ATT	CGT	CTA	TCA	CGT	1344
	Gln	Tyr	Ser	Phe	Leu	Leu	Lys	Leu	Asn	Lys	Ala	Ile	Arg	Leu	Ser	Arg	
			435				440						445				
30	GCG	ACA	GAA	TTG	TCA	CCC	ACG	ATT	CTG	GAA	GGC	ATT	GTG	CGC	AGT	GTT	1392
	Ala	Thr	Glu	Leu	Ser	Pro	Thr	Ile	Leu	Glu	Gly	Ile	Val	Arg	Ser	Val	
	450						455					460					
35	AAT	CTA	CAA	CTG	GAT	ATC	AAC	ACA	GAC	GTA	TTA	GGT	AAA	GTT	TTT	CTG	1440
	Asn	Leu	Gln	Leu	Asp	Ile	Asn	Thr	Asp	Val	Leu	Gly	Lys	Val	Phe	Leu	
	465				470					475						480	
40	ACT	AAA	TAT	TAT	ATG	CAG	CGT	TAT	GCT	ATT	CAT	GCT	GAA	ACT	GCC	CTG	1488
	Thr	Lys	Tyr	Tyr	Met	Gln	Arg	Tyr	Ala	Ile	His	Ala	Glu	Thr	Ala	Leu	
					485				490						495		
45	ATA	CTA	TGC	AAC	GCG	CCT	ATT	TCA	CAA	CGT	TCA	TAT	GAT	AAT	CAA	CCT	1536
	Ile	Leu	Cys	Asn	Ala	Pro	Ile	Ser	Gln	Arg	Ser	Tyr	Asp	Asn	Gln	Pro	
				500				505						510			
50	AGC	CAA	TTT	GAT	CGC	CTG	TTT	AAT	ACG	CCA	TTA	CTG	AAC	GGA	CAA	TAT	1584
	Ser	Gln	Phe	Asp	Arg	Leu	Phe	Asn	Thr	Pro	Leu	Leu	Asn	Gly	Gln	Tyr	
			515				520						525				
55	TTT	TCT	ACC	GGC	GAT	GAG	GAG	ATT	GAT	TTA	AAT	TCA	GGT	AGC	ACC	GGC	1632
	Phe	Ser	Thr	Gly	Asp	Glu	Glu	Ile	Asp	Leu	Asn	Ser	Gly	Ser	Thr	Gly	
	530					535						540					
60	GAT	TGG	CGA	AAA	ACC	ATA	CTT	AAG	CGT	GCA	TTT	AAT	ATT	GAT	GAT	GTC	1680
	Asp	Trp	Arg	Lys	Thr	Ile	Leu	Lys	Arg	Ala	Phe	Asn	Ile	Asp	Asp	Val	
	545				550					555						560	
65	TCG	CTC	TTC	CGC	CTG	CTT	AAA	ATT	ACC	GAC	CAT	GAT	AAT	AAA	GAT	GGA	1728
	Ser	Leu	Phe	Arg	Leu	Leu	Lys	Ile	Thr	Asp	His	Asp	Asn	Lys	Asp	Gly	
				565					570						575		
70	AAA	ATT	AAA	AAT	AAC	CTA	AAG	AAT	CTT	TCC	AAT	TTA	TAT	ATT	GGA	AAA	1776
	Lys	Ile	Lys	Asn	Asn	Leu	Lys	Asn	Leu	Ser	Asn	Leu	Tyr	Ile	Gly	Lys	
				580				585						590			
75	TTA	CTG	GCA	GAT	ATT	CAT	CAA	TTA	ACC	ATT	GAT	GAA	CTG	GAT	TTA	TTA	1824
	Leu	Leu	Ala	Asp	Ile	His	Gln	Leu	Thr	Ile	Asp	Glu	Leu	Asp	Leu	Leu	
			595				600					605					
80	CTG	ATT	GCC	GTA	GGT	GAA	GGA	AAA	ACT	AAT	TTA	TCC	GCT	ATC	AGT	GAT	1872
	Leu	Ile	Ala	Val	Gly	Glu	Gly	Lys	Thr	Asn	Leu	Ser	Ala	Ile	Ser	Asp	
	610					615						620					
85	AAG	CAA	TTG	GCT	ACC	CTG	ATC	AGA	AAA	CTC	AAT	ACT	ATT	ACC	AGC	TGG	1920
	Lys	Gln	Leu	Ala	Thr	Leu	Ile	Arg	Lys	Leu	Asn	Thr	Ile	Thr	Ser	Trp	
	625				630					635						640	
90	CTA	CAT	ACA	CAG	AAG	TGG	AGT	GTA	TTC	CAG	CTA	TTT	ATC	ATG	ACC	TCC	1968
	Leu	His	Thr	Gln	Lys	Trp	Ser	Val	Phe	Gln	Leu	Phe	Ile	Met	Thr	Ser	

	645								650								655								
5	ACC Thr	AGC Ser	TAT Tyr	AAC Asn 660	AAA Lys	ACG Thr	CTA Leu	ACG Thr	CCT Pro 665	GAA Glu	ATT Ile	AAG Lys	AAT Asn	TTG Leu 670	CTG Leu	GAT Asp	2016								
	ACC Thr	GTC Val	TAC Tyr 675	CAC His	GGT Gly	TTA Leu	CAA Gln	GGT Gly 680	TTT Phe	GAT Asp	AAA Lys	GAC Asp	AAA Lys 685	GCA Ala	GAT Asp	TTG Leu	2064								
10	CTA Leu	CAT His 690	GTC Val	ATG Met	GCG Ala	CCC Pro	TAT Tyr 695	ATT Ile	GCG Ala	GCC Ala	ACC Thr	TTG Leu 700	CAA Gln	TTA Leu	TCA Ser	TCG Ser	2112								
15	GAA Glu 705	AAT Asn	GTC Val	GCC Ala	CAC His	TCG Ser 710	GTA Val	CTC Leu	CTT Leu	TGG Trp	GCA Ala 715	GAT Asp	AAG Lys	TTA Leu	CAG Gln	CCC Pro 720	2160								
20	GGC Gly	GAC Asp	GGC Gly	GCA Ala	ATG Met 725	ACA Thr	GCA Ala	GAA Glu	AAA Lys	TTC Phe 730	TGG Trp	GAC Asp	TGG Trp	TTG Leu 735	AAT Asn	ACT Thr	2208								
25	AAG Lys	TAT Tyr	ACG Thr	CCG Pro 740	GGT Gly	TCA Ser	TCG Ser	GAA Glu	GCC Ala 745	GTA Val	GAA Glu	ACG Thr	CAG Gln	GAA Glu 750	CAT His	ATC Ile	2256								
	GTT Val	CAG Gln	TAT Tyr 755	TGT Cys	CAG Gln	GCT Ala	CTG Leu	GCA Ala 760	CAA Gln	TTG Leu	GAA Glu	ATG Met	GTT Val 765	TAC Tyr	CAT His	TCC Ser	2304								
30	ACC Thr	GGC Gly 770	ATC Ile	AAC Asn	GAA Glu	AAC Asn	GCC Ala 775	TTC Phe	CGT Arg	CTA Leu	TTT Phe	GTG Val 780	ACA Thr	AAA Lys	CCA Pro	GAG Glu	2352								
35	ATG Met 785	TTT Phe	GGC Gly	GCT Ala	GCA Ala	ACT Thr 790	GGA Gly	GCA Ala	GCG Ala	CCC Pro	GCG Ala 795	CAT His	GAT Asp	GCC Ala	CTT Leu	TCA Ser 800	2400								
40	CTG Leu	ATT Ile	ATG Met	CTG Leu	ACA Thr 805	CGT Arg	TTT Phe	GCG Ala	GAT Asp	TGG Trp 810	GTG Val	AAC Asn	GCA Ala	CTA Leu	GGC Gly 815	GAA Glu	2448								
45	AAA Lys	GCG Ala	TCC Ser	TCG Ser 820	GTG Val	CTA Leu	GCG Ala	GCA Ala	TTT Phe 825	GAA Glu	GCT Ala	AAC Asn	TCG Ser	TTA Leu 830	ACG Thr	GCA Ala	2496								
	GAA Glu	CAA Gln	CTG Leu 835	GCT Ala	GAT Asp	GCC Ala	ATG Met	AAT Asn 840	CTT Leu	GAT Asp	GCT Ala	AAT Asn	TTG Leu 845	CTG Leu	TTG Leu	CAA Gln	2544								
50	GCC Ala	AGT Ser 850	ATT Ile	CAA Gln	GCA Ala	CAA Gln	AAT Asn 855	CAT His	CAA Gln	CAT His	CTT Leu	CCC Pro 860	CCA Pro	GTA Val	ACT Thr	CCA Pro	2592								
55	GAA Glu 865	AAT Asn	GCG Ala	TTC Phe	TCC Ser	TGT Cys 870	TGG Trp	ACA Thr	TCT Ser	ATC Ile	AAT Asn 875	ACT Thr	ATC Ile	CTG Leu	CAA Gln	TGG Trp 880	2640								
60	GTT Val	AAT Asn	GTC Val	GCA Ala	CAA Gln 885	CAA Gln	TTG Leu	AAT Asn	GTC Val	GCC Ala 890	CCA Pro	CAG Gln	GGC Gly	GTT Val 895	TCC Ser	GCT Ala	2688								
65	TTG Leu	GTC Val	GGG Gly	CTG Leu 900	GAT Asp	TAT Tyr	ATT Ile	CAA Gln	TCA Ser 905	ATG Met	AAA Lys	GAG Glu	ACA Thr	CCG Pro 910	ACC Thr	TAT Tyr	2736								
	GCC Ala	CAG Gln	TGG Trp 915	GAA Glu	AAC Asn	GCG Ala	GCA Ala	GGC Gly 920	GTA Val	TTA Leu	ACC Thr	GCC Ala	GGG Gly 925	TTG Leu	AAT Asn	TCA Ser	2784								
70	CAA Glu	CAG Gln	GCT Trp	AAT Asp	ACA Gln	TTA Leu	CAC Gln	GCT Trp	TTT Phe	CTG Leu	GAT Asp	GAA Glu	TCT Thr	CGC Pro	AGT Thr	GCC Pro	2832								

	Gln	Gln	Ala	Asn	Thr	Leu	His	Ala	Phe	Leu	Asp	Glu	Ser	Arg	Ser	Ala	
	930						935					940					
5	GCA	TTA	AGC	ACC	TAC	TAT	ATC	CGT	CAA	GTC	GCC	AAG	GCA	GCG	GCG	GCT	2880
	Ala	Leu	Ser	Thr	Tyr	Tyr	Ile	Arg	Gln	Val	Ala	Lys	Ala	Ala	Ala	Ala	
	945					950					955					960	
10	ATT	AAA	AGC	CGT	GAT	GAC	TTG	TAT	CAA	TAC	TTA	CTG	ATT	GAT	AAT	CAG	2928
	Ile	Lys	Ser	Arg		Asp	Leu	Tyr	Gln	Tyr	Leu	Leu	Ile	Asp	Asn	Gln	
				965						970					975		
15	GTT	TCT	GCG	GCA	ATA	AAA	ACC	ACC	CGG	ATC	GCC	GAA	GCC	ATT	GCC	AGT	2976
	Val	Ser	Ala	Ala	Ile	Lys	Thr	Thr	Arg	Ile	Ala	Glu	Ala	Ile	Ala	Ser	
				980					985					990			
20	ATT	CAA	CTG	TAC	GTC	AAC	CGG	GCA	TTG	GAA	AAT	GTG	GAA	GAA	AAT	GCC	3024
	Ile	Gln	Leu	Tyr	Val	Asn	Arg	Ala	Leu	Glu	Asn	Val	Glu	Glu	Asn	Ala	
			995					1000					1005				
25	AAT	TCG	GGG	GTT	ATC	AGC	CGC	CAA	TTC	TTT	ATC	GAC	TGG	GAC	AAA	TAC	3072
	Asn	Ser	Gly	Val	Ile	Ser	Arg	Gln	Phe	Phe	Ile	Asp	Trp	Asp	Lys	Tyr	
		1010					1015					1020					
30	AAT	AAA	CGC	TAC	AGC	ACT	TGG	GCG	GGT	GTT	TCT	CAA	TTA	GTT	TAC	TAC	3120
	Asn	Lys	Arg	Tyr	Ser	Thr	Trp	Ala	Gly	Val	Ser	Gln	Leu	Val	Tyr	Tyr	
		1025				1030					1035					1040	
35	CCG	GAA	AAC	TAT	ATT	GAT	CCG	ACC	ATG	CGT	ATC	GGA	CAA	ACC	AAA	ATG	3168
	Pro	Glu	Asn	Tyr	Ile	Asp	Pro	Thr	Met	Arg	Ile	Gly	Gln	Thr	Lys	Met	
				1045						1050					1055		
40	ATG	GAC	GCA	TTA	CTG	CAA	TCC	GTC	AGC	CAA	AGC	CAA	TTA	AAC	GCC	GAT	3216
	Met	Asp	Ala	Leu	Leu	Gln	Ser	Val	Ser	Gln	Ser	Gln	Leu	Asn	Ala	Asp	
				1060					1065					1070			
45	ACC	GTC	GAA	GAT	GCC	TTT	ATG	TCT	TAT	CTG	ACA	TCG	TTT	GAA	CAA	GTG	3264
	Thr	Val	Glu	Asp	Ala	Phe	Met	Ser	Tyr	Leu	Thr	Ser	Phe	Glu	Gln	Val	
			1075				1080						1085				
50	GCT	AAT	CTT	AAA	GTT	ATT	AGC	GCA	TAT	CAC	GAT	AAT	ATT	AAT	AAC	GAT	3312
	Ala	Asn	Leu	Lys	Val	Ile	Ser	Ala	Tyr	His	Asp	Asn	Ile	Asn	Asn	Asp	
		1090				1095						1100					
55	CAA	GGG	CTG	ACC	TAT	TTT	ATC	GGA	CTC	AGT	GAA	ACT	GAT	GCC	GGT	GAA	3360
	Gln	Gly	Leu	Thr	Tyr	Phe	Ile	Gly	Leu	Ser	Glu	Thr	Asp	Ala	Gly	Glu	
		1105				1110					1115					1120	
60	TAT	TAT	TGG	CGC	AGT	GTC	GAT	CAC	AGT	AAA	TTC	AAC	GAC	GGT	AAA	TTC	3408
	Tyr	Tyr	Trp	Arg	Ser	Val	Asp	His	Ser	Lys	Phe	Asn	Asp	Gly	Lys	Phe	
				1125						1130					1135		
65	GCG	GCT	AAT	GCC	TGG	AGT	GAA	TGG	CAT	AAA	ATT	GAT	TGT	CCA	ATT	AAC	3456
	Ala	Ala	Asn	Ala	Trp	Ser	Glu	Trp	His	Lys	Ile	Asp	Cys	Pro	Ile	Asn	
				1140					1145					1150			
70	CCT	TAT	AAA	AGC	ACT	ATC	CGT	CCA	GTG	ATA	TAT	AAA	TCC	CGC	CTG	TAT	3504
	Pro	Tyr	Lys	Ser	Thr	Ile	Arg	Pro	Val	Ile	Tyr	Lys	Ser	Arg	Leu	Tyr	
			1155					1160					1165				
75	CTG	CTC	TGG	TTG	GAA	CAA	AAG	GAG	ATC	ACC	AAA	CAG	ACA	GGA	AAT	AGT	3552
	Leu	Leu	Trp	Leu	Glu	Gln	Lys	Glu	Ile	Thr	Lys	Gln	Thr	Gly	Asn	Ser	
			1170				1175					1180					
80	AAA	GAT	GGC	TAT	CAA	ACT	GAA	ACG	GAT	TAT	CGT	TAT	GAA	CTA	AAA	TTG	3600
	Lys	Asp	Gly	Tyr	Gln	Thr	Glu	Thr	Asp	Tyr	Arg	Tyr	Glu	Leu	Lys	Leu	
		1185				1190					1195					1200	
85	GCG	CAT	ATC	CGC	TAT	GAT	GGC	ACT	TGG	AAT	ACG	CCA	ATC	ACC	TTT	GAT	3648
	Ala	His	Ile	Arg	Tyr	Asp	Gly	Thr	Trp	Asn	Thr	Pro	Ile	Thr	Phe	Asp	
				1205						1210					1215		

	GTC	AAT	AAA	AAA	ATA	TCC	GAG	CTA	AAA	CTG	GAA	AAA	AAT	AGA	GCG	CCC	3636
	Val	Asn	Lys	Lys	Ile	Ser	Glu	Leu	Lys	Leu	Glu	Lys	Asn	Arg	Ala	Pro	
				1220					1225					1230			
5	GGA	CTC	TAT	TGT	GCC	GGT	TAT	CAA	GGT	GAA	GAT	ACG	TTG	CTG	GTG	ATG	3744
	Gly	Leu	Tyr	Cys	Ala	Gly	Tyr	Gln	Gly	Glu	Asp	Thr	Leu	Leu	Val	Met	
				1235				1240					1245				
10	TTT	TAT	AAC	CAA	CAA	GAC	ACA	CTA	GAT	AGT	TAT	AAA	AAC	GCT	TCA	ATG	3792
	Phe	Tyr	Asn	Gln	Gln	Asp	Thr	Leu	Asp	Ser	Tyr	Lys	Asn	Ala	Ser	Met	
			1250				1255					1260					
15	CAA	GGA	CTA	TAT	ATC	TTT	GCT	GAT	ATG	GCA	TCC	AAA	GAT	ATG	ACC	CCA	3840
	Gln	Gly	Leu	Tyr	Ile	Phe	Ala	Asp	Met	Ala	Ser	Lys	Asp	Met	Thr	Pro	
			1265			1270				1275						1280	
20	GAA	CAG	AGC	AAT	GTT	TAT	CGG	GAT	AAT	AGC	TAT	CAA	CAA	TTT	GAT	ACC	3888
	Glu	Gln	Ser	Asn	Val	Tyr	Arg	Asp	Asn	Ser	Tyr	Gln	Gln	Phe	Asp	Thr	
				1285						1290					1295		
25	AAT	AAT	GTC	AGA	AGA	GTG	AAT	AAC	CGC	TAT	GCA	GAG	GAT	TAT	GAG	ATT	3936
	Asn	Asn	Val	Arg	Arg	Val	Asn	Asn	Arg	Tyr	Ala	Glu	Asp	Tyr	Glu	Ile	
				1300					1305						1310		
30	CCT	TCC	TCG	GTA	AGT	AGC	CGT	AAA	GAC	TAT	GGT	TGG	GGA	GAT	TAT	TAC	3984
	Pro	Ser	Ser	Val	Ser	Ser	Arg	Lys	Asp	Tyr	Gly	Trp	Gly	Asp	Tyr	Tyr	
				1315				1320					1325				
35	CTC	AGC	ATG	GTA	TAT	AAC	GGA	GAT	ATT	CCA	ACT	ATC	AAT	TAC	AAA	GCC	4032
	Leu	Ser	Met	Val	Tyr	Asn	Gly	Asp	Ile	Pro	Thr	Ile	Asn	Tyr	Lys	Ala	
			1330				1335					1340					
40	GCA	TCA	AGT	GAT	TTA	AAA	ATC	TAT	ATC	TCA	CCA	AAA	TTA	AGA	ATT	ATT	4080
	Ala	Ser	Ser	Asp	Leu	Lys	Ile	Tyr	Ile	Ser	Pro	Lys	Leu	Arg	Ile	Ile	
				1345		1350				1355						1360	
45	CAT	AAT	GGA	TAT	GAA	GGA	CAG	AAG	CGC	AAT	CAA	TGC	AAT	CTG	ATG	AAT	4128
	His	Asn	Gly	Tyr	Glu	Gly	Gln	Lys	Arg	Asn	Gln	Cys	Asn	Leu	Met	Asn	
					1365				1370						1375		
50	AAA	TAT	GGC	AAA	CTA	GGT	GAT	AAA	TTT	ATT	GTT	TAT	ACT	AGC	TTG	GGG	4176
	Lys	Tyr	Gly	Lys	Leu	Gly	Asp	Lys	Phe	Ile	Val	Tyr	Thr	Ser	Leu	Gly	
				1380				1385						1390			
55	GTC	AAT	CCA	AAT	AAC	TCG	TCA	AAT	AAG	CTC	ATG	TTT	TAC	CCC	GTC	TAT	4224
	Val	Asn	Pro	Asn	Asn	Ser	Ser	Asn	Lys	Leu	Met	Phe	Tyr	Pro	Val	Tyr	
				1395				1400					1405				
60	CAA	TAT	AGC	GGA	AAC	ACC	AGT	GGA	CTC	AAT	CAA	GGG	AGA	CTA	CTA	TTC	4272
	Gln	Tyr	Ser	Gly	Asn	Thr	Ser	Gly	Leu	Asn	Gln	Gly	Arg	Leu	Leu	Phe	
				1410			1415					1420					
65	CAC	CGT	GAC	ACC	ACT	TAT	CCA	TCT	AAA	GTA	GAA	GCT	TGG	ATT	CCT	GGA	4320
	His	Arg	Asp	Thr	Thr	Tyr	Pro	Ser	Lys	Val	Glu	Ala	Trp	Ile	Pro	Gly	
				1425		1430				1435						1440	
70	GCA	AAA	CGT	TCT	CTA	ACC	AAC	CAA	AAT	GCC	GCC	ATT	GGT	GAT	GAT	TAT	4368
	Ala	Lys	Arg	Ser	Leu	Thr	Asn	Gln	Asn	Ala	Ala	Ile	Gly	Asp	Asp	Tyr	
					1445					1450					1455		
75	GCT	ACA	GAC	TCT	CTG	AAT	AAA	CCG	GAT	GAT	CTT	AAG	CAA	TAT	ATC	TTT	4416
	Ala	Thr	Asp	Ser	Leu	Asn	Lys	Pro	Asp	Asp	Leu	Lys	Gln	Tyr	Ile	Phe	
				1460				1465						1470			
80	ATG	ACT	GAC	AGT	AAA	GGG	ACT	GCT	ACT	GAT	GTC	TCA	GGC	CCA	GTA	GAG	4464
	Met	Thr	Asp	Ser	Lys	Gly	Thr	Ala	Thr	Asp	Val	Ser	Gly	Pro	Val	Glu	
				1475			1480					1485					
85	ATT	AAT	ACT	GCA	ATT	TCT	CCA	GCA	AAA	GTT	CAG	ATA	ATA	GTC	AAA	GCG	4512
	Ile	Asn	Thr	Ala	Ile	Ser	Pro	Ala	Lys	Val	Gln	Ile	Ile	Val	Lys	Ala	
				1490			1495					1500					

5	GGT GGC AAG GAG CAA ACT TTT ACC GCA GAT AAA GAT GTC TCC ATT CAG 4560
	Gly Gly Lys Glu Gln Thr Phe Thr Ala Asp Lys Asp Val Ser Ile Gln 1505 1510 1515 1520
10	CCA TCA CCT AGC TTT GAT GAA ATG AAT TAT CAA TTT AAT GCC CTT GAA 4608
	Pro Ser Pro Ser Phe Asp Glu Met Asn Tyr Gln Phe Asn Ala Leu Glu 1525 1530 1535
15	ATA GAC GGT TCT GGT CTG AAT TTT ATT AAC AAC TCA GCC AGT ATT GAT 4656
	Ile Asp Gly Ser Gly Leu Asn Phe Ile Asn Asn Ser Ala Ser Ile Asp 1540 1545 1550
20	GTT ACT TTT ACC GCA TTT GCG GAG GAT GGC CGC AAA CTG GGT TAT GAA 4704
	Val Thr Phe Thr Ala Phe Ala Glu Asp Gly Arg Lys Leu Gly Tyr Glu 1555 1560 1565
25	AGT TTC AGT ATT CCT GTT ACC CTC AAG GTA AGT ACC GAT AAT GCC CTG 4752
	Ser Phe Ser Ile Pro Val Thr Leu Lys Val Ser Thr Asp Asn Ala Leu 1570 1575 1580
30	ACC CTG CAC CAT AAT GAA AAT GGT GCG CAA TAT ATG CAA TGG CAA TCC 4800
	Thr Leu His His Asn Glu Asn Gly Ala Gln Tyr Met Gln Trp Gln Ser 1585 1590 1595 1600
35	TAT CGT ACC CGC CTG AAT ACT CTA TTT GCC CGC CAG TTG GTT GCA CGC 4848
	Tyr Arg Thr Arg Leu Asn Thr Leu Phe Ala Arg Gln Leu Val Ala Arg 1605 1610 1615
40	GCC ACC ACC GGA ATC GAT ACA ATT CTG AGT ATG GAA ACT CAG AAT ATT 4896
	Ala Thr Thr Gly Ile Asp Thr Ile Leu Ser Met Glu Thr Gln Asn Ile 1620 1625 1630
45	CAG GAA CCG CAG TTA GGC AAA GGT TTC TAT GCT ACG TTC GTG ATA CCT 4944
	Gln Glu Pro Gln Leu Gly Lys Gly Phe Tyr Ala Thr Phe Val Ile Pro 1635 1640 1645
50	CCC TAT AAC CTA TCA ACT CAT GGT GAT GAA CGT TGG TTT AAG CTT TAT 4992
	Pro Tyr Asn Leu Ser Thr His Gly Asp Glu Arg Trp Phe Lys Leu Tyr 1650 1655 1660
55	ATC AAA CAT GTT GTT GAT AAT AAT TCA CAT ATT ATC TAT TCA GGC CAG 5040
	Ile Lys His Val Val Asp Asn Asn Ser His Ile Ile Tyr Ser Gly Gln 1665 1670 1675 1680
60	CTA ACA GAT ACA AAT ATA AAC ATC ACA TTA TTT ATT CCT CTT GAT GAT 5088
	Leu Thr Asp Thr Asn Ile Asn Ile Thr Leu Phe Ile Pro Leu Asp Asp 1685 1690 1695
65	GTC CCA TTG AAT CAA GAT TAT CAC GCC AAG GTT TAT ATG ACC TTC AAG 5136
	Val Pro Leu Asn Gln Asp Tyr His Ala Lys Val Tyr Met Thr Phe Lys 1700 1705 1710
70	AAA TCA CCA TCA GAT GGT ACC TGG TGG GGC CCT CAC TTT GTT AGA GAT 5184
	Lys Ser Pro Ser Asp Gly Thr Trp Trp Gly Pro His Phe Val Arg Asp 1715 1720 1725
75	GAT AAA GGA ATA GTA ACA ATA AAC CCT AAA TCC ATT TTG ACC CAT TTT 5232
	Asp Lys Gly Ile Val Thr Ile Asn Pro Lys Ser Ile Leu Thr His Phe 1730 1735 1740
80	GAG AGC GTC AAT GTC CTG AAT AAT ATT AGT AGC GAA CCA ATG GAT TTC 5280
	Glu Ser Val Asn Val Leu Asn Asn Ile Ser Ser Glu Pro Met Asp Phe 1745 1750 1755 1760
85	AGC GGC GCT AAC AGC CTC TAT TTC TGG GAA CTG TTC TAC TAT ACC CCG 5328
	Ser Gly Ala Asn Ser Leu Tyr Phe Trp Glu Leu Phe Tyr Tyr Thr Pro 1765 1770 1775
90	ATG CTG GTT GCT CAA CGT TTG CTG CAT GAA CAG AAC TTC GAT GAA GCC 5376
	Met Leu Val Ala Gln Arg Leu Leu His Glu Gln Asn Phe Asp Glu Ala 1780 1785 1790

	1730	1785	1790	
5	AAC CGT TGG CTG AAA TAT GTC Asn Arg Trp Leu Lys Tyr Val	TGG AGT CCA TCC GGT Trp Ser Pro Ser Gly Tyr	TAT ATT GTC CAC Ile Val His	5424
	1795	1800	1805	
10	GGC CAG ATT CAG AAC TAC CAG Gly Gln Ile Gln Asn Tyr	TGG AAC GTC CGC CCG TTA Trp Asn Val Arg Pro Leu	CTG GAA GAC Leu Leu Glu Asp	5472
	1810	1815	1820	
	ACC AGT TGG AAC AGT GAT CCT Thr Ser Trp Asn Ser Pro	TTG GAT TCC GTC GAT CCT Leu Asp Ser Val Asp Pro	GAC GCG GTA Asp Ala Val	5520
	1825	1830	1835	1840
15	GCA CAG CAC GAT CCA ATG CAC Ala Gln His Asp Pro Met	TAC AAA GTT TCA ACT TTT His Tyr Lys Val Ser Thr	ATG CGT ACC Met Arg Thr	5568
	1845	1850	1855	
20	TTG GAT CTA TTG ATA GCA CGC Leu Asp Leu Ile Ala Arg	GGC GAC CAT GCT TAT CGC Gly Asp His Ala Tyr Arg	CAA CTG GAA Gln Leu Glu	5616
	1860	1865	1870	
25	CGA GAT ACA CTC AAC GAA GCG Arg Asp Thr Leu Asn Glu	AAG ATG TGG TAT ATG CAA Lys Met Trp Tyr Met Gln	CTG CAT Ala Leu His	5664
	1875	1880	1885	
30	CTA TTA GGT GAC AAA CCT Leu Leu Gly Asp Lys Pro	TAT CTA CCG CTG AGT ACG Tyr Leu Pro Leu Ser Thr	ACA TGG AGT GAT Thr Thr Trp Ser Asp	5712
	1890	1895	1900	
	CCA CGA CTA GAC AGA GCC Pro Arg Leu Asp Arg Ala	GCG GAT ATC ACT ACC CAA Ala Ala Asp Ile Thr Thr	AAT GCT CAC GAC Asn Ala His Asp	5760
	1905	1910	1915	1920
35	AGC GCA ATA GTC GCT CTG CGG Ser Ala Ile Val Ala Leu	CAG AAT ATA CCT ACA CCG Gln Asn Ile Pro Thr Pro	GCA CCT TTA Ala Pro Leu	5808
	1925	1930	1935	
40	TCA TTG CGC AGC GCT AAT ACC Ser Leu Arg Ser Ala Asn	CTG ACT GAT CTC TTC CTG Leu Thr Asp Leu Phe Leu	CCG CAA ATC Pro Gln Ile	5856
	1940	1945	1950	
45	AAT GAA GTG ATG ATG AAT TAC Asn Glu Val Met Met Asn	TGG CAG ACA TTA GCT CAG Trp Gln Thr Leu Ala Gln	AGA GTA TAC Arg Val Tyr	5904
	1955	1960	1965	
	AAT CTG CGT CAT AAC CTC Asn Leu Arg His Asn Leu	TCT ATC GAC GGC CAG CCG Ser Ile Asp Gly Gln Pro	TTA TAT CTG CCA Leu Tyr Leu Pro	5952
	1970	1975	1980	
50	ATC TAT GCC ACA CCG GCC Ile Tyr Ala Thr Pro Ala	GAT CCG AAA GCG TTA CTC Asp Pro Lys Ala Leu Leu	AGC GCC GCC GTT Ser Ala Ala Val	6000
	1985	1990	1995	2000
55	GCC ACT TCT CAA GGT GGA GGC Ala Thr Ser Gln Gly Gly	AAG CTA CCG GAA TCA TTT Lys Leu Pro Glu Ser Phe	ATG TCC CTG Met Ser Leu	6048
	2005	2010	2015	
60	TGG CGT TTC CCG CAC ATG CTG Trp Arg Phe Pro His Met	GAA AAT GCG CGC GGC ATG Glu Asn Ala Arg Gly Met	GTT AGC CAG Val Ser Gln	6096
	2020	2025	2030	
65	CTC ACC CAG TTC GGC TCC ACG Leu Thr Gln Phe Gly Ser	TTA CAA AAT ATT ATC GAA Leu Gln Asn Ile Ile Glu	CGT CAG GAC Arg Gln Asp	6144
	2035	2040	2045	
70	GCG GAA GCG CTC AAT GCG Ala Glu Ala Leu Asn Ala	TTA TTA CAA AAT CAG GCC Leu Leu Gln Asn Gln Ala	GCC GAG CTG ATA Ala Glu Leu Ile	6192
	2050	2055	2060	
	TTG ACT AAC CTG AGC ATT CAG Trp Arg Phe Pro His Met	GAC AAA ACC ATT GAA GAA Leu Thr Asp Leu Phe Leu	TTG GAT GCC Trp Ser Gln	6240

	Leu	Thr	Asn	Leu	Ser	Ile	Gln	Asp	Lys	Thr	Ile	Glu	Glu	Leu	Asp	Ala	
	2065					2070				2075					2080		
5	GAG	AAA	ACG	GTG	TTG	GAA	AAA	TCC	AAA	GCG	GGA	GCA	CAA	TCG	CGC	TTT	6283
	Glu	Lys	Thr	Val	Leu	Glu	Lys	Ser	Lys	Ala	Gly	Ala	Gln	Ser	Arg	Phe	
					2085					2090					2095		
10	GAT	AGC	TAC	GGC	AAA	CTG	TAC	GAT	GAG	AAT	ATC	AAC	GCC	GGT	GAA	AAC	6336
	Asp	Ser	Tyr	Gly	Lys	Leu	Tyr	Asp	Glu	Asn	Ile	Asn	Ala	Gly	Glu	Asn	
				2100					2105					2110			
15	CAA	GCC	ATG	ACG	CTA	CGA	GCG	TCC	GCC	GCC	GGG	CTT	ACC	ACG	GCA	GTT	6384
	Gln	Ala	Met	Thr	Leu	Arg	Ala	Ser	Ala	Ala	Gly	Leu	Thr	Thr	Ala	Val	
				2115				2120					2125				
20	CAG	GCA	TCC	CGT	CTG	GCC	GGT	GCG	GCG	GCT	GAT	CTG	GTG	CCT	AAC	ATC	6432
	Gln	Ala	Ser	Arg	Leu	Ala	Gly	Ala	Ala	Ala	Asp	Leu	Val	Pro	Asn	Ile	
		2130					2135					2140					
25	TTC	GGC	TTT	GCC	GGT	GGC	GGC	AGC	CGT	TGG	GGG	GCT	ATC	GCT	GAG	GCG	6480
	Phe	Gly	Phe	Ala	Gly	Gly	Gly	Ser	Arg	Trp	Gly	Ala	Ile	Ala	Glu	Ala	
		2145				2150					2155				2160		
30	ACA	GGT	TAT	GTG	ATG	GAA	TTC	TCC	GCG	AAT	GTT	ATG	AAC	ACC	GAA	GCG	6528
	Thr	Gly	Tyr	Val	Met	Glu	Phe	Ser	Ala	Asn	Val	Met	Asn	Thr	Glu	Ala	
					2165					2170					2175		
35	GAT	AAA	ATT	AGC	CAA	TCT	GAA	ACC	TAC	CGT	CGT	CGC	CGT	CAG	GAG	TGG	6576
	Asp	Lys	Ile	Ser	Gln	Ser	Glu	Thr	Tyr	Arg	Arg	Arg	Arg	Gln	Glu	Trp	
				2180					2185					2190			
40	GAG	ATC	CAG	CGG	AAT	AAT	GCC	GAA	GCG	GAA	TTG	AAG	CAA	ATC	GAT	GCT	6624
	Glu	Ile	Gln	Arg	Asn	Asn	Ala	Glu	Ala	Glu	Leu	Lys	Gln	Ile	Asp	Ala	
				2195			2200						2205				
45	CAG	CTC	AAA	TCA	CTC	GCT	GTA	CGC	CGC	GAA	GCC	GCC	GTA	TTG	CAG	AAA	6672
	Gln	Leu	Lys	Ser	Leu	Ala	Val	Arg	Arg	Glu	Ala	Ala	Val	Leu	Gln	Lys	
		2210					2215					2220					
50	ACC	AGT	CTG	AAA	ACC	CAA	CAA	GAA	CAG	ACC	CAA	TCT	CAA	TTG	GCC	TTC	6720
	Thr	Ser	Leu	Lys	Thr	Gln	Gln	Glu	Gln	Thr	Gln	Ser	Gln	Leu	Ala	Phe	
		2225				2230					2235				2240		
55	CTG	CAA	CGT	AAG	TTC	AGC	AAT	CAG	GCG	TTA	TAC	AAC	TGG	CTG	CGT	GGT	6768
	Leu	Gln	Arg	Lys	Phe	Ser	Asn	Gln	Ala	Leu	Tyr	Asn	Trp	Leu	Arg	Gly	
					2245				2250					2255			
60	CGA	CTG	GCG	GCG	ATT	TAC	TTC	CAG	TTC	TAC	GAT	TTG	GCC	GTC	GCG	CGT	6816
	Arg	Leu	Ala	Ala	Ile	Tyr	Phe	Gln	Phe	Tyr	Asp	Leu	Ala	Val	Ala	Arg	
				2260				2265					2270				
65	TGC	CTG	ATG	GCA	GAA	CAA	GCT	TAC	CGT	TGG	GAA	CTC	AAT	GAT	GAC	TCT	6864
	Cys	Leu	Met	Ala	Glu	Gln	Ala	Tyr	Arg	Trp	Glu	Leu	Asn	Asp	Asp	Ser	
			2275				2280					2285					
70	GCC	CGC	TTC	ATT	AAA	CCG	GGC	GCC	TGG	CAG	GGA	ACC	TAT	GCC	GGT	CTG	6912
	Ala	Arg	Phe	Ile	Lys	Pro	Gly	Ala	Trp	Gln	Gly	Thr	Tyr	Ala	Gly	Leu	
		2290					2295				2300						
75	CTT	GCA	GGT	GAA	ACC	TTG	ATG	CTG	AGT	CTG	GCA	CAA	ATG	GAA	GAC	GCT	6960
	Leu	Ala	Gly	Glu	Thr	Leu	Met	Leu	Ser	Leu	Ala	Gln	Met	Glu	Asp	Ala	
		2305				2310					2315				2320		
80	CAT	CTG	AAA	CGC	GAT	AAA	CGC	GCA	TTA	GAG	GTT	GAA	CGC	ACA	GTA	TCG	7008
	His	Leu	Lys	Arg	Asp	Lys	Arg	Ala	Leu	Glu	Val	Glu	Arg	Thr	Val	Ser	
				2325					2330					2335			
85	CTG	GCC	GAA	GTT	TAT	GCA	GGA	TTA	CCA	AAA	GAT	AAC	GGT	CCA	TTT	TCC	7056
	Leu	Ala	Glu	Val	Tyr	Ala	Gly	Leu	Pro	Lys	Asp	Asn	Gly	Pro	Phe	Ser	
				2340				2345					2350				



CTG GGT CAG GAA ATT GAC AAG CTG GTG AGT CAA GGT TCA GGC AGT GCC 7194  
 Leu Ala Gln Glu Ile Asp Lys Leu Val Ser Gln Gly Ser Gly Ser Ala  
 2355 2360 2365  
 5 GGC AGT GGT AAT AAT AAT TTG GCG TTC GGC GCC GGC ACG GAC ACT AAA 7192  
 Gly Ser Gly Asn Asn Asn Leu Ala Phe Gly Ala Gly Thr Asp Thr Lys  
 2370 2375 2380  
 10 ACC TCT TTG CAG GCA TCA GTT TCA TTC GCT GAT TTG AAA ATT CGT GAA 7200  
 Thr Ser Leu Gln Ala Ser Val Ser Phe Ala Asp Leu Lys Ile Arg Glu  
 2385 2390 2395 2400  
 15 GAT TAC CCG GCA TCG CTT GGC AAA ATT CGA CGT ATC AAA CAG ATC AGC 7248  
 Asp Tyr Pro Ala Ser Leu Gly Lys Ile Arg Arg Ile Lys Gln Ile Ser  
 2405 2410 2415  
 20 GTC ACT TTG CCC GCG CTA CTG GGA CCG TAT CAG GAT GTA CAG GCA ATA 7296  
 Val Thr Leu Pro Ala Leu Leu Gly Pro Tyr Gln Asp Val Gln Ala Ile  
 2420 2425 2430  
 TTG TCT TAC GGC GAT AAA GCC GGA TTA GCT AAC GGC TGT GAA GCG CTG 7344  
 Leu Ser Tyr Gly Asp Lys Ala Gly Leu Ala Asn Gly Cys Glu Ala Leu  
 2435 2440 2445  
 25 GCA GTT TCT CAC GGT ATG AAT GAC AGC GGC CAA TTC CAG CTC GAT TTC 7392  
 Ala Val Ser His Gly Met Asn Asp Ser Gly Gln Phe Gln Leu Asp Phe  
 2450 2455 2460  
 30 AAC GAT GGC AAA TTC CTG CCA TTC GAA GGC ATC GCC ATT GAT CAA GGC 7440  
 Asn Asp Gly Lys Phe Leu Pro Phe Glu Gly Ile Ala Ile Asp Gln Gly  
 2465 2470 2475 2480  
 35 ACG CTG ACA CTG AGC TTC CCA AAT GCA TCT ATG CCG GAG AAA GGT AAA 7488  
 Thr Leu Thr Leu Ser Phe Pro Asn Ala Ser Met Pro Glu Lys Gly Lys  
 2485 2490 2495  
 40 CAA GCC ACT ATG TTA AAA ACC CTG AAC GAT ATC ATT TTG CAT ATT CGC 7536  
 Gln Ala Thr Met Leu Lys Thr Leu Asn Asp Ile Ile Leu His Ile Arg  
 2500 2505 2510  
 TAC ACC ATT AAA TAA 7551  
 Tyr Thr Ile Lys ...  
 2516

45

## (2) INFORMATION FOR SEQ ID NO:47:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 2516 amino acids  
 (B) TYPE: amino acids  
 50 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

55

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:47 (TcdA):

Features	From	To	Description
Peptide	1	2516	TcdA proteins
Peptide	89	1937	TcdA <sub>11</sub> peptide
Fragment	89	100	S2 N-terminus (SEQ ID NO:13)
60 Fragment	284	299	(SEQ ID NO:38)
Fragment	554	563	(SEQ ID NO:17)
Fragment	1080	1092	(SEQ ID NO:23; 12/13)
Fragment	1385	1400	(SEQ ID NO:18)
Fragment	1473	1497	(SEQ ID NO:39)
65 Fragment	1620	1642	(SEQ ID NO:21; 19/23)
Fragment	1938	1948	(SEQ ID NO:41)
Peptide	1938	2516	TcdA <sub>11</sub> peptide
Fragment	2327	2345	(SEQ ID NO:42)
Fragment	2398	2408	(SEQ ID NO:43)

	Met	Asn	Glu	Ser	Val	Lys	Glu	Ile	Pro	Asp	Val	Leu	Lys	Ser	Gln	Cys	
	1				5					10					15		
5	Gly	Phe	Asn	Cys	Leu	Thr	Asp	Ile	Ser	His	Ser	Ser	Phe	Asn	Glu	Phe	
				20					25					30			
	Arg	Gln	Gln	Val	Ser	Glu	His	Leu	Ser	Trp	Ser	Glu	Thr	His	Asp	Leu	
			35					40					45				
10	Tyr	His	Asp	Ala	Gln	Gln	Ala	Gln	Lys	Asp	Asn	Arg	Leu	Tyr	Glu	Ala	
		50					55					60					
	Arg	Ile	Leu	Lys	Arg	Ala	Asn	Pro	Gln	Leu	Gln	Asn	Ala	Val	His	Leu	
15		65				70					75					80	
	Ala	Ile	Leu	Ala	Pro	Asn	Ala	Glu	Leu	Ile	Gly	Tyr	Asn	Asn	Gln	Phe	
					85					90					95		
20	Ser	Gly	Arg	Ala	Ser	Gln	Tyr	Val	Ala	Pro	Gly	Thr	Val	Ser	Ser	Met	
				100					105					110			
	Phe	Ser	Pro	Ala	Ala	Tyr	Leu	Thr	Glu	Leu	Tyr	Arg	Glu	Ala	Arg	Asn	
			115				120						125				
25	Leu	His	Ala	Ser	Asp	Ser	Val	Tyr	Tyr	Leu	Asp	Thr	Arg	Arg	Pro	Asp	
		130					135					140					
	Leu	Lys	Ser	Met	Ala	Leu	Ser	Gln	Gln	Asn	Met	Asp	Ile	Glu	Leu	Ser	
30		145				150					155					160	
	Thr	Leu	Ser	Leu	Ser	Asn	Glu	Leu	Leu	Leu	Glu	Ser	Ile	Lys	Thr	Glu	
				165						170					175		
35	Ser	Lys	Leu	Glu	Asn	Tyr	Thr	Lys	Val	Met	Glu	Met	Leu	Ser	Thr	Phe	
				180					185					190			
	Arg	Pro	Ser	Gly	Ala	Thr	Pro	Tyr	His	Asp	Ala	Tyr	Glu	Asn	Val	Arg	
			195					200					205				
40	Glu	Val	Ile	Gln	Leu	Gln	Asp	Pro	Gly	Leu	Glu	Gln	Leu	Asn	Ala	Ser	
		210					215					220					
	Pro	Ala	Ile	Ala	Gly	Leu	Met	His	Gln	Ala	Ser	Leu	Leu	Gly	Ile	Asn	
45		225				230					235					240	
	Ala	Ser	Ile	Ser	Pro	Glu	Leu	Phe	Asn	Ile	Leu	Thr	Glu	Glu	Ile	Thr	
				245						250					255		
50	Glu	Gly	Asn	Ala	Glu	Glu	Leu	Tyr	Lys	Lys	Asn	Phe	Gly	Asn	Ile	Glu	
				260					265					270			
	Pro	Ala	Ser	Leu	Ala	Met	Pro	Glu	Tyr	Leu	Lys	Arg	Tyr	Tyr	Asn	Leu	
			275				280						285				
55	Ser	Asp	Glu	Glu	Leu	Ser	Gln	Phe	Ile	Gly	Lys	Ala	Ser	Asn	Phe	Gly	
		290					295					300					
	Gln	Gln	Glu	Tyr	Ser	Asn	Asn	Gln	Leu	Ile	Thr	Pro	Val	Val	Asn	Ser	
60		305				310					315					320	
	Ser	Asp	Gly	Thr	Val	Lys	Val	Tyr	Arg	Ile	Thr	Arg	Glu	Tyr	Thr	Thr	
				325						330					335		
65	Asn	Ala	Tyr	Gln	Met	Asp	Val	Glu	Leu	Phe	Pro	Phe	Gly	Gly	Glu	Asn	
				340				345					350				
	Tyr	Arg	Leu	Asp	Tyr	Lys	Phe	Lys	Asn	Phe	Tyr	Asn	Ala	Ser	Tyr	Leu	
			355				360					365					
70	Ser	Ile	Lys	Leu	Asn	Asp	Lys	Arg	Glu	Leu	Val	Arg	Thr	Glu	Gly	Ala	

		370				375					380						
		Pro	Gln	Val	Asn	Ile	Glu	Tyr	Ser	Ala	Asn	Ile	Thr	Leu	Asn	Thr	Ala
		385					390					395					400
5		Asp	Ile	Ser	Gln	Pro	Phe	Glu	Ile	Gly	Leu	Thr	Arg	Val	Leu	Pro	Ser
					405						410					415	
10		Gly	Ser	Trp	Ala	Tyr	Ala	Ala	Ala	Lys	Phe	Thr	Val	Glu	Glu	Tyr	Asn
					420					425					430		
		Gln	Tyr	Ser	Phe	Leu	Leu	Lys	Leu	Asn	Lys	Ala	Ile	Arg	Leu	Ser	Arg
				435					440					445			
15		Ala	Thr	Glu	Leu	Ser	Pro	Thr	Ile	Leu	Glu	Gly	Ile	Val	Arg	Ser	Val
		450						455					460				
		Asn	Leu	Gln	Leu	Asp	Ile	Asn	Thr	Asp	Val	Leu	Gly	Lys	Val	Phe	Leu
		465					470					475					480
20		Thr	Lys	Tyr	Tyr	Met	Gln	Arg	Tyr	Ala	Ile	His	Ala	Glu	Thr	Ala	Leu
						485					490					495	
25		Ile	Leu	Cys	Asn	Ala	Pro	Ile	Ser	Gln	Arg	Ser	Tyr	Asp	Asn	Gln	Pro
					500					505					510		
		Ser	Gln	Phe	Asp	Arg	Leu	Phe	Asn	Thr	Pro	Leu	Leu	Asn	Gly	Gln	Tyr
				515					520					525			
30		Phe	Ser	Thr	Gly	Asp	Glu	Glu	Ile	Asp	Leu	Asn	Ser	Gly	Ser	Thr	Gly
		530						535						540			
		Asp	Trp	Arg	Lys	Thr	Ile	Leu	Lys	Arg	Ala	Phe	Asn	Ile	Asp	Asp	Val
		545					550					555					560
35		Ser	Leu	Phe	Arg	Leu	Leu	Lys	Ile	Thr	Asp	His	Asp	Asn	Lys	Asp	Gly
					565						570				575		
40		Lys	Ile	Lys	Asn	Asn	Leu	Lys	Asn	Leu	Ser	Asn	Leu	Tyr	Ile	Gly	Lys
					580					585					590		
		Leu	Leu	Ala	Asp	Ile	His	Gln	Leu	Thr	Ile	Asp	Glu	Leu	Asp	Leu	Leu
				595					600					605			
45		Leu	Ile	Ala	Val	Gly	Glu	Gly	Lys	Thr	Asn	Leu	Ser	Ala	Ile	Ser	Asp
		610						615					620				
		Lys	Gln	Leu	Ala	Thr	Leu	Ile	Arg	Lys	Leu	Asn	Thr	Ile	Thr	Ser	Trp
		625					630					635					640
50		Leu	His	Thr	Gln	Lys	Trp	Ser	Val	Phe	Gln	Leu	Phe	Ile	Met	Thr	Ser
					645						650					655	
55		Thr	Ser	Tyr	Asn	Lys	Thr	Leu	Thr	Pro	Glu	Ile	Lys	Asn	Leu	Leu	Asp
					660					665					670		
		Thr	Val	Tyr	His	Gly	Leu	Gln	Gly	Phe	Asp	Lys	Asp	Lys	Ala	Asp	Leu
				675					680					685			
60		Leu	His	Val	Met	Ala	Pro	Tyr	Ile	Ala	Ala	Thr	Leu	Gln	Leu	Ser	Ser
		690						695					700				
		Glu	Asn	Val	Ala	His	Ser	Val	Leu	Leu	Trp	Ala	Asp	Lys	Leu	Gln	Pro
		705					710					715					720
65		Gly	Asp	Gly	Ala	Met	Thr	Ala	Glu	Lys	Phe	Trp	Asp	Trp	Leu	Asn	Thr
					725						730					735	
70		Lys	Tyr	Thr	Pro	Gly	Ser	Ser	Glu	Ala	Val	Glu	Thr	Gln	Glu	His	Ile
					740					745					750		

Val Gln Tyr Cys Gln Ala Leu Ala Gln Leu Glu Met Val Tyr His Ser  
 755 760 765  
 5 Thr Gly Ile Asn Glu Asn Ala Phe Arg Leu Phe Val Thr Lys Pro Glu  
 770 775 780  
 Met Phe Gly Ala Ala Thr Gly Ala Ala Pro Ala His Asp Ala Leu Ser  
 785 790 795 800  
 10 Leu Ile Met Leu Thr Arg Phe Ala Asp Trp Val Asn Ala Leu Gly Glu  
 805 810 815  
 Lys Ala Ser Ser Val Leu Ala Ala Phe Glu Ala Asn Ser Leu Thr Ala  
 820 825 830  
 15 Glu Gln Leu Ala Asp Ala Met Asn Leu Asp Ala Asn Leu Leu Gln  
 835 840 845  
 Ala Ser Ile Gln Ala Gln Asn His Gln His Leu Pro Pro Val Thr Pro  
 850 855 860  
 20 Glu Asn Ala Phe Ser Cys Trp Thr Ser Ile Asn Thr Ile Leu Gln Trp  
 865 870 875 880  
 25 Val Asn Val Ala Gln Gln Leu Asn Val Ala Pro Gln Gly Val Ser Ala  
 885 890 895  
 Leu Val Gly Leu Asp Tyr Ile Gln Ser Met Lys Glu Thr Pro Thr Tyr  
 900 905 910  
 30 Ala Gln Trp Glu Asn Ala Ala Gly Val Leu Thr Ala Gly Leu Asn Ser  
 915 920 925  
 Gln Gln Ala Asn Thr Leu His Ala Phe Leu Asp Glu Ser Arg Ser Ala  
 930 935 940  
 35 Ala Leu Ser Thr Tyr Tyr Ile Arg Gln Val Ala Lys Ala Ala Ala Ala  
 945 950 955 960  
 40 Ile Lys Ser Arg Asp Asp Leu Tyr Gln Tyr Leu Leu Ile Asp Asn Gln  
 965 970 975  
 Val Ser Ala Ala Ile Lys Thr Thr Arg Ile Ala Glu Ala Ile Ala Ser  
 980 985 990  
 45 Ile Gln Leu Tyr Val Asn Arg Ala Leu Glu Asn Val Glu Glu Asn Ala  
 995 1000 1005  
 Asn Ser Gly Val Ile Ser Arg Gln Phe Phe Ile Asp Trp Asp Lys Tyr  
 1010 1015 1020  
 50 Asn Lys Arg Tyr Ser Thr Trp Ala Gly Val Ser Gln Leu Val Tyr Tyr  
 1025 1030 1035 1040  
 55 Pro Glu Asn Tyr Ile Asp Pro Thr Met Arg Ile Gly Gln Thr Lys Met  
 1045 1050 1055  
 Met Asp Ala Leu Leu Gln Ser Val Ser Gln Ser Gln Leu Asn Ala Asp  
 1060 1065 1070  
 60 Thr Val Glu Asp Ala Phe Met Ser Tyr Leu Thr Ser Phe Glu Gln Val  
 1075 1080 1085  
 Ala Asn Leu Lys Val Ile Ser Ala Tyr His Asp Asn Ile Asn Asn Asp  
 1090 1095 1100  
 65 Gln Gly Leu Thr Tyr Phe Ile Gly Leu Ser Glu Thr Asp Ala Gly Glu  
 1105 1110 1115 1120  
 70 Tyr Tyr Trp Arg Ser Val Asp His Ser Lys Phe Asn Asp Gly Lys Phe  
 1125 1130 1135

Ala Ala Asn Ala Trp Ser Glu Trp His Lys Ile Asp Cys Pro Ile Asn  
1140 1145 1150

5 Pro Tyr Lys Ser Thr Ile Arg Pro Val Ile Tyr Lys Ser Arg Leu Tyr  
1155 1160 1165

Leu Leu Trp Leu Glu Gln Lys Glu Ile Thr Lys Gln Thr Gly Asn Ser  
1170 1175 1180

10 Lys Asp Gly Tyr Gln Thr Glu Thr Asp Tyr Arg Tyr Glu Leu Lys Leu  
1185 1190 1195 1200

Ala His Ile Arg Tyr Asp Gly Thr Trp Asn Thr Pro Ile Thr Phe Asp  
1205 1210 1215

15 Val Asn Lys Lys Ile Ser Glu Leu Lys Leu Glu Lys Asn Arg Ala Pro  
1220 1225 1230

20 Gly Leu Tyr Cys Ala Gly Tyr Gln Gly Glu Asp Thr Leu Leu Val Met  
1235 1240 1245

Phe Tyr Asn Gln Gln Asp Thr Leu Asp Ser Tyr Lys Asn Ala Ser Met  
1250 1255 1260

25 Gln Gly Leu Tyr Ile Phe Ala Asp Met Ala Ser Lys Asp Met Thr Pro  
1265 1270 1275 1280

30 Glu Gln Ser Asn Val Tyr Arg Asp Asn Ser Tyr Gln Gln Phe Asp Thr  
1285 1290 1295

Asn Asn Val Arg Arg Val Asn Asn Arg Tyr Ala Glu Asp Tyr Glu Ile  
1300 1305 1310

35 Pro Ser Ser Val Ser Ser Arg Lys Asp Tyr Gly Trp Gly Asp Tyr Tyr  
1315 1320 1325

Leu Ser Met Val Tyr Asn Gly Asp Ile Pro Thr Ile Asn Tyr Lys Ala  
1330 1335 1340

40 Ala Ser Ser Asp Leu Lys Ile Tyr Ile Ser Pro Lys Leu Arg Ile Ile  
1345 1350 1355 1360

His Asn Gly Tyr Glu Gly Gln Lys Arg Asn Gln Cys Asn Leu Met Asn  
1365 1370 1375

45 Lys Tyr Gly Lys Leu Gly Asp Lys Phe Ile Val Tyr Thr Ser Leu Gly  
1380 1385 1390

50 Val Asn Pro Asn Asn Ser Ser Asn Lys Leu Met Phe Tyr Pro Val Tyr  
1395 1400 1405

Gln Tyr Ser Gly Asn Thr Ser Gly Leu Asn Gln Gly Arg Leu Leu Phe  
1410 1415 1420

55 His Arg Asp Thr Thr Tyr Pro Ser Lys Val Glu Ala Trp Ile Pro Gly  
1425 1430 1435 1440

Ala Lys Arg Ser Leu Thr Asn Gln Asn Ala Ala Ile Gly Asp Asp Tyr  
1445 1450 1455

60 Ala Thr Asp Ser Leu Asn Lys Pro Asp Asp Leu Lys Gln Tyr Ile Phe  
1460 1465 1470

65 Met Thr Asp Ser Lys Gly Thr Ala Thr Asp Val Ser Gly Pro Val Glu  
1475 1480 1485

Ile Asn Thr Ala Ile Ser Pro Ala Lys Val Gln Ile Ile Val Lys Ala  
1490 1495 1500

70 Gly Gly Lys Glu Gln Thr Phe Thr Ala Asp Lys Asp Val Ser Ile Gln

	1505		1510		1515		1520
	Pro Ser Pro Ser Phe Asp Glu Met Asn Tyr Gln Phe Asn Ala Leu Glu						
		1525			1530		1535
5	Ile Asp Gly Ser Gly Leu Asn Phe Ile Asn Asn Ser Ala Ser Ile Asp						
		1540			1545		1550
10	Val Thr Phe Thr Ala Phe Ala Glu Asp Gly Arg Lys Leu Gly Tyr Glu						
		1555			1560		1565
	Ser Phe Ser Ile Pro Val Thr Leu Lys Val Ser Thr Asp Asn Ala Leu						
		1570			1575		1580
15	Thr Leu His His Asn Glu Asn Gly Ala Gln Tyr Met Gln Trp Gln Ser						
			1590			1595	1600
	Tyr Arg Thr Arg Leu Asn Thr Leu Phe Ala Arg Gln Leu Val Ala Arg						
		1605			1610		1615
20	Ala Thr Thr Gly Ile Asp Thr Ile Leu Ser Met Glu Thr Gln Asn Ile						
		1620			1625		1630
25	Gln Glu Pro Gln Leu Gly Lys Gly Phe Tyr Ala Thr Phe Val Ile Pro						
		1635			1640		1645
	Pro Tyr Asn Leu Ser Thr His Gly Asp Glu Arg Trp Phe Lys Leu Tyr						
		1650			1655		1660
30	Ile Lys His Val Val Asp Asn Asn Ser His Ile Ile Tyr Ser Gly Gln						
			1670			1675	1680
	Leu Thr Asp Thr Asn Ile Asn Ile Thr Leu Phe Ile Pro Leu Asp Asp						
		1685			1690		1695
35	Val Pro Leu Asn Gln Asp Tyr His Ala Lys Val Tyr Met Thr Phe Lys						
		1700			1705		1710
40	Lys Ser Pro Ser Asp Gly Thr Trp Trp Gly Pro His Phe Val Arg Asp						
		1715			1720		1725
	Asp Lys Gly Ile Val Thr Ile Asn Pro Lys Ser Ile Leu Thr His Phe						
		1730			1735		1740
45	Glu Ser Val Asn Val Leu Asn Asn Ile Ser Ser Glu Pro Met Asp Phe						
			1750			1755	1760
	Ser Gly Ala Asn Ser Leu Tyr Phe Trp Glu Leu Phe Tyr Tyr Thr Pro						
		1765			1770		1775
50	Met Leu Val Ala Gln Arg Leu Leu His Glu Gln Asn Phe Asp Glu Ala						
		1780			1785		1790
55	Asn Arg Trp Leu Lys Tyr Val Trp Ser Pro Ser Gly Tyr Ile Val His						
		1795			1800		1805
	Gly Gln Ile Gln Asn Tyr Gln Trp Asn Val Arg Pro Leu Leu Glu Asp						
		1810			1815		1820
60	Thr Ser Trp Asn Ser Asp Pro Leu Asp Ser Val Asp Pro Asp Ala Val						
			1830			1835	1840
	Ala Gln His Asp Pro Met His Tyr Lys Val Ser Thr Phe Met Arg Thr						
		1845			1850		1855
65	Leu Asp Leu Leu Ile Ala Arg Gly Asp His Ala Tyr Arg Gln Leu Glu						
		1860			1865		1870
70	Arg Asp Thr Leu Asn Glu Ala Lys Met Trp Tyr Met Gln Ala Leu His						
		1875			1880		1885

	Leu	Leu	Gly	Asp	Lys	Pro	Tyr	Leu	Pro	Leu	Ser	Thr	Thr	Trp	Ser	Asp	
	1390						1895					1900					
5	Pro	Arg	Leu	Asp	Arg	Ala	Ala	Asp	Ile	Thr	Thr	Gln	Asn	Ala	His	Asp	
	1905					1910					1915					1920	
	Ser	Ala	Ile	Val	Ala	Leu	Arg	Gln	Asn	Ile	Pro	Thr	Pro	Ala	Pro	Leu	
					1925					1930					1935		
10	Ser	Leu	Arg	Ser	Ala	Asn	Thr	Leu	Thr	Asp	Leu	Phe	Leu	Pro	Gln	Ile	
				1940					1945					1950			
	Asn	Glu	Val	Met	Met	Asn	Tyr	Trp	Gln	Thr	Leu	Ala	Gln	Arg	Val	Tyr	
			1955					1960					1965				
15	Asn	Leu	Arg	His	Asn	Leu	Ser	Ile	Asp	Gly	Gln	Pro	Leu	Tyr	Leu	Pro	
	1970						1975					1980					
20	Ile	Tyr	Ala	Thr	Pro	Ala	Asp	Pro	Lys	Ala	Leu	Leu	Ser	Ala	Ala	Val	
	1985					1990					1995					2000	
	Ala	Thr	Ser	Gln	Gly	Gly	Gly	Lys	Leu	Pro	Glu	Ser	Phe	Met	Ser	Leu	
					2005					2010					2015		
25	Trp	Arg	Phe	Pro	His	Met	Leu	Glu	Asn	Ala	Arg	Gly	Met	Val	Ser	Gln	
				2020					2025					2030			
	Leu	Thr	Gln	Phe	Gly	Ser	Thr	Leu	Gln	Asn	Ile	Ile	Glu	Arg	Gln	Asp	
			2035					2040					2045				
30	Ala	Glu	Ala	Leu	Asn	Ala	Leu	Leu	Gln	Asn	Gln	Ala	Ala	Glu	Leu	Ile	
		2050					2055					2060					
35	Leu	Thr	Asn	Leu	Ser	Ile	Gln	Asp	Lys	Thr	Ile	Glu	Glu	Leu	Asp	Ala	
	2065					2070					2075					2080	
	Glu	Lys	Thr	Val	Leu	Glu	Lys	Ser	Lys	Ala	Gly	Ala	Gln	Ser	Arg	Phe	
					2085					2090					2095		
40	Asp	Ser	Tyr	Gly	Lys	Leu	Tyr	Asp	Glu	Asn	Ile	Asn	Ala	Gly	Glu	Asn	
				2100					2105					2110			
	Gln	Ala	Met	Thr	Leu	Arg	Ala	Ser	Ala	Ala	Gly	Leu	Thr	Thr	Ala	Val	
			2115					2120					2125				
45	Gln	Ala	Ser	Arg	Leu	Ala	Gly	Ala	Ala	Ala	Asp	Leu	Val	Pro	Asn	Ile	
		2130					2135					2140					
50	Phe	Gly	Phe	Ala	Gly	Gly	Gly	Ser	Arg	Trp	Gly	Ala	Ile	Ala	Glu	Ala	
	2145				2150						2155					2160	
	Thr	Gly	Tyr	Val	Met	Glu	Phe	Ser	Ala	Asn	Val	Met	Asn	Thr	Glu	Ala	
					2165					2170					2175		
55	Asp	Lys	Ile	Ser	Gln	Ser	Glu	Thr	Tyr	Arg	Arg	Arg	Arg	Gln	Glu	Trp	
				2180					2185					2190			
	Glu	Ile	Gln	Arg	Asn	Asn	Ala	Glu	Ala	Glu	Leu	Lys	Gln	Ile	Asp	Ala	
			2195					2200					2205				
60	Gln	Leu	Lys	Ser	Leu	Ala	Val	Arg	Arg	Glu	Ala	Ala	Val	Leu	Gln	Lys	
		2210					2215					2220					
65	Thr	Ser	Leu	Lys	Thr	Gln	Gln	Glu	Gln	Thr	Gln	Ser	Gln	Leu	Ala	Phe	
	2225					2230					2235					2240	
	Leu	Gln	Arg	Lys	Phe	Ser	Asn	Gln	Ala	Leu	Tyr	Asn	Trp	Leu	Arg	Gly	
					2245					2250					2255		
70	Arg	Leu	Ala	Ala	Ile	Tyr	Phe	Gln	Phe	Tyr	Asp	Leu	Ala	Val	Ala	Arg	
				2260					2265					2270			

Cys Leu Met Ala Glu Gln Ala Tyr Arg Trp Glu Leu Asn Asp Asp Ser  
 2275 2280 2285  
 5 Ala Arg Phe Ile Lys Pro Gly Ala Trp Gln Gly Thr Tyr Ala Gly Leu  
 2290 2295 2300  
 Leu Ala Gly Glu Thr Leu Met Leu Ser Leu Ala Gln Met Glu Asp Ala  
 2305 2310 2315 2320  
 10 His Leu Lys Arg Asp Lys Arg Ala Leu Glu Val Glu Arg Thr Val Ser  
 2325 2330 2335  
 Leu Ala Glu Val Tyr Ala Gly Leu Pro Lys Asp Asn Gly Pro Phe Ser  
 2340 2345 2350  
 15 Leu Ala Gln Glu Ile Asp Lys Leu Val Ser Gln Gly Ser Gly Ser Ala  
 2355 2360 2365  
 Gly Ser Gly Asn Asn Asn Leu Ala Phe Gly Ala Gly Thr Asp Thr Lys  
 2370 2375 2380  
 Thr Ser Leu Gln Ala Ser Val Ser Phe Ala Asp Leu Lys Ile Arg Glu  
 2385 2390 2395 2400  
 25 Asp Tyr Pro Ala Ser Leu Gly Lys Ile Arg Arg Ile Lys Gln Ile Ser  
 2405 2410 2415  
 Val Thr Leu Pro Ala Leu Leu Gly Pro Tyr Gln Asp Val Gln Ala Ile  
 2420 2425 2430  
 30 Leu Ser Tyr Gly Asp Lys Ala Gly Leu Ala Asn Gly Cys Glu Ala Leu  
 2435 2440 2445  
 Ala Val Ser His Gly Met Asn Asp Ser Gly Gln Phe Gln Leu Asp Phe  
 2450 2455 2460  
 Asn Asp Gly Lys Phe Leu Pro Phe Glu Gly Ile Ala Ile Asp Gln Gly  
 2465 2470 2475 2480  
 40 Thr Leu Thr Leu Ser Phe Pro Asn Ala Ser Met Pro Glu Lys Gly Lys  
 2485 2490 2495  
 Gln Ala Thr Met Leu Lys Thr Leu Asn Asp Ile Ile Leu His Ile Arg  
 2500 2505 2510  
 45 Tyr Thr Ile Lys  
 2516

50

## (2) INFORMATION FOR SEQ ID NO:48:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5547 base pairs  
 (B) TYPE: nucleic acid  
 55 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

60

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48 (tcdA<sub>ii</sub> coding region):

CTG ATA GGC TAT AAC AAT CAA TTT AGC GGT AGA GCC AGT CAA TAT GTT 48  
 Leu Ile Gly Tyr Asn Asn Gln Phe Ser Gly Arg Ala Ser Gln Tyr Val  
 1 5 10 15  
 GCG CCG GGT ACC GTT TCT TCC ATG TTC TCC CCC GCC GCT TAT TTG ACT 96  
 Ala Pro Gly Thr Val Ser Ser Met Phe Ser Pro Ala Ala Tyr Leu Thr  
 20 25 30



GAA CTT TAT CGT GAA GCA CGC AAT TTA CAC GCA AGT GAC TCC GTT TAT 144  
 Glu Leu Tyr Arg Glu Ala Arg Asn Leu His Ala Ser Asp Ser Val Tyr  
 35 40 45  
 5 TAT CTG GAT ACC CGC CGC CCA GAT CTC AAA TCA ATG GCG CTC AGT CAG 192  
 Tyr Leu Asp Thr Arg Arg Pro Asp Leu Lys Ser Met Ala Leu Ser Gln  
 50 55 60  
 10 CAA AAT ATG GAT ATA GAA TTA TCC ACA CTC TCT TTG TCC AAT GAG CTG 240  
 Gln Asn Met Asp Ile Glu Leu Ser Thr Leu Ser Leu Ser Asn Glu Leu  
 65 70 75 80  
 15 TTA TTG GAA AGC ATT AAA ACT GAA TCT AAA CTG GAA AAC TAT ACT AAA 288  
 Leu Leu Glu Ser Ile Lys Thr Glu Ser Lys Leu Glu Asn Tyr Thr Lys  
 85 90 95  
 20 GTG ATG GAA ATG CTC TCC ACT TTC CGT CCT TCC GGC GCA ACG CCT TAT 336  
 Val Met Glu Met Leu Ser Thr Phe Arg Pro Ser Gly Ala Thr Pro Tyr  
 100 105 110  
 25 CAT GAT GCT TAT GAA AAT GTG CGT GAA GTT ATC CAG CTA CAA GAT CCT 384  
 His Asp Ala Tyr Glu Asn Val Arg Glu Val Ile Gln Leu Gln Asp Pro  
 115 120 125  
 GGA CTT GAG CAA CTC AAT GCA TCA CCG GCA ATT GCC GGG TTG ATG CAT 432  
 Gly Leu Glu Gln Leu Asn Ala Ser Pro Ala Ile Ala Gly Leu Met His  
 130 135 140  
 30 CAA GCC TCC CTA TTG GGT AAT AAC GCT TCA ATC TCG CCT GAG CTA TTT 480  
 Gln Ala Ser Leu Leu Gly Ile Asn Ala Ser Ile Ser Pro Glu Leu Phe  
 145 150 155 160  
 35 AAT ATT CTG ACG GAG GAG ATT ACC GAA GGT AAT GCT GAG GAA CTT TAT 528  
 Asn Ile Leu Thr Glu Glu Ile Thr Glu Gly Asn Ala Glu Glu Leu Tyr  
 165 170 175  
 40 AAG AAA AAT TTT GGT AAT ATC GAA CCG GCC TCA TTG GCT ATG CCG GAA 576  
 Lys Lys Asn Phe Gly Asn Ile Glu Pro Ala Ser Leu Ala Met Pro Glu  
 180 185 190  
 TAC CTT AAA CGT TAT TAT AAT TTA AGC GAT GAA GAA CTT AGT CAG TTT 624  
 Tyr Leu Lys Arg Tyr Tyr Asn Leu Ser Asp Glu Glu Leu Ser Gln Phe  
 195 200 205  
 45 ATT GGT AAA GCC AGC AAT TTT GGT CAA CAG GAA TAT AGT AAT AAC CAA 672  
 Ile Gly Lys Ala Ser Asn Phe Gly Gln Gln Glu Tyr Ser Asn Asn Gln  
 210 215 220  
 50 CTT ATT ACT CCG GTA GTC AAC AGC AGT GAT GGC ACG GTT AAG GTA TAT 720  
 Leu Ile Thr Pro Val Val Asn Ser Ser Asp Gly Thr Val Lys Val Tyr  
 225 230 235 240  
 55 CGG ATC ACC CGC GAA TAT ACA ACC AAT GCT TAT CAA ATG GAT GTG GAG 768  
 Arg Ile Thr Arg Glu Tyr Thr Thr Asn Ala Tyr Gln Met Asp Val Glu  
 245 250 255  
 CTA TTT CCC TTC GGT GGT GAG AAT TAT CGG TTA GAT TAT AAA TTC AAA 816  
 Leu Phe Pro Phe Gly Gly Glu Asn Tyr Arg Leu Asp Tyr Lys Phe Lys  
 260 265 270  
 60 AAT TTT TAT AAT GCC TCT TAT TTA TCC ATC AAG TTA AAT GAT AAA AGA 864  
 Asn Phe Tyr Asn Ala Ser Tyr Leu Ser Ile Lys Leu Asn Asp Lys Arg  
 275 280 285  
 65 GAA CTT GTT CGA ACT GAA GGC GCT CCT CAA GTC AAT ATA GAA TAC TCC 912  
 Glu Leu Val Arg Thr Glu Gly Ala Pro Gln Val Asn Ile Glu Tyr Ser  
 290 295 300  
 70 GCA AAT ATC ACA TTA AAT ACC GCT GAT ATC AGT CAA CCT TTT GAA ATT 960  
 Ala Asn Ile Thr Leu Asn Thr Ala Asp Ile Ser Gln Pro Phe Glu Ile

	305		310		315		320	
5	GGC CTG ACA CGA GTA CTT CCT TCC GGT TCT TGG GCA TAT GCC GCC GCA 1068 Gly Leu Thr Arg Val Leu Pro Ser Gly Ser Trp Ala Tyr Ala Ala Ala	325		330		335		
10	AAA TTT ACC GTT GAA GAG TAT AAC CAA TAC TCT TTT CTG CTA AAA CTT 1056 Lys Phe Thr Val Glu Glu Tyr Asn Gln Tyr Ser Phe Leu Leu Lys Leu	340		345		350		
15	AAC AAG GCT ATT CGT CTA TCA CGT GCG ACA GAA TTG TCA CCC ACG ATT 1104 Asn Lys Ala Ile Arg Leu Ser Arg Ala Thr Glu Leu Ser Pro Thr Ile	355		360		365		
20	CTG GAA GGC ATT GTG CGC AGT GTT AAT CTA CAA CTG GAT ATC AAC ACA 1152 Leu Glu Gly Ile Val Arg Ser Val Asn Leu Gln Leu Asp Ile Asn Thr	370		375		380		
25	GAC GTA TTA GGT AAA GTT TTT CTG ACT AAA TAT TAT ATG CAG CGT TAT 1200 Asp Val Leu Gly Lys Val Phe Leu Thr Lys Tyr Tyr Met Gln Arg Tyr	385		390		395		400
30	GCT ATT CAT GCT GAA ACT GCC CTG ATA CTA TGC AAC GCG CCT ATT TCA 1248 Ala Ile His Ala Glu Thr Ala Leu Ile Leu Cys Asn Ala Pro Ile Ser	405		410		415		
35	CAA CGT TCA TAT GAT AAT CAA CCT AGC CAA TTT GAT CGC CTG TTT AAT 1296 Gln Arg Ser Tyr Asp Asn Gln Pro Ser Gln Phe Asp Arg Leu Phe Asn	420		425		430		
40	ACG CCA TTA CTG AAC GGA CAA TAT TTT TCT ACC GGC GAT GAG GAG ATT 1344 Thr Pro Leu Leu Asn Gly Gln Tyr Phe Ser Thr Gly Asp Glu Glu Ile	435		440		445		
45	GAT TTA AAT TCA GGT AGC ACC GGC GAT TGG CGA AAA ACC ATA CTT AAG 1392 Asp Leu Asn Ser Gly Ser Thr Gly Asp Trp Arg Lys Thr Ile Leu Lys	450		455		460		
50	CGT GCA TTT AAT ATT GAT GAT GTC TCG CTC TTC CGC CTG CTT AAA ATT 1440 Arg Ala Phe Asn Ile Asp Asp Val Ser Leu Phe Arg Leu Leu Lys Ile	465		470		475		480
55	ACC GAC CAT GAT AAT AAA GAT GGA AAA ATT AAA AAT AAC CTA AAG AAT 1488 Thr Asp His Asp Asn Lys Asp Gly Lys Ile Lys Asn Asn Leu Lys Asn	485		490		495		
60	CTT TCC AAT TTA TAT ATT GGA AAA TTA CTG GCA GAT ATT CAT CAA TTA 1536 Leu Ser Asn Leu Tyr Ile Gly Lys Leu Leu Ala Asp Ile His Gln Leu	500		505		510		
65	ACC ATT GAT GAA CTG GAT TTA TTA CTG ATT GCC GTA GGT GAA GGA AAA 1584 Thr Ile Asp Glu Leu Asp Leu Leu Leu Ile Ala Val Gly Glu Gly Lys	515		520		525		
70	ACT AAT TTA TCC GCT ATC AGT GAT AAG CAA TTG GCT ACC CTG ATC AGA 1632 Thr Asn Leu Ser Ala Ile Ser Asp Lys Gln Leu Ala Thr Leu Ile Arg	530		535		540		
75	AAA CTC AAT ACT ATT ACC AGC TGG CTA CAT ACA CAG AAG TGG AGT GTA 1680 Lys Leu Asn Thr Ile Thr Ser Trp Leu His Thr Gln Lys Trp Ser Val	545		550		555		560
80	TTC CAG CTA TTT ATC ATG ACC TCC ACC AGC TAT AAC AAA ACG CTA ACG 1728 Phe Gln Leu Phe Ile Met Thr Ser Thr Ser Tyr Asn Lys Thr Leu Thr	565		570		575		
85	CCT GAA ATT AAG AAT TTG CTG GAT ACC GTC TAC CAC GGT TTA CAA GGT 1776 Pro Glu Ile Lys Asn Leu Leu Asp Thr Val Tyr His Gly Leu Gln Gly	580		585		590		
90	TTT GAT AAA GAC AAA GCA GAT TTG CTA CAT GTC ATG GCG CCC TAT ATT 1824							

	Phe	Asp	Lys	Asp	Lys	Ala	Asp	Leu	Leu	His	Val	Met	Ala	Pro	Tyr	Ile	
			595					600					605				
5	GCG	GCC	ACC	TTG	CAA	TTA	TCA	TCG	GAA	AAT	GTC	GCC	CAC	TCG	GTA	CTC	1872
	Ala	Ala	Thr	Leu	Gln	Leu	Ser	Ser	Glu	Asn	Val	Ala	His	Ser	Val	Leu	
			610				615					620					
10	CTT	TGG	GCA	GAT	AAG	TTA	CAG	CCC	GGC	GAC	GGC	GCA	ATG	ACA	GCA	GAA	1920
	Leu	Trp	Ala	Asp	Lys	Leu	Gln	Pro	Gly	Asp	Gly	Ala	Met	Thr	Ala	Glu	
	625					630					635					640	
15	AAA	TTC	TGG	GAC	TGG	TTG	AAT	ACT	AAG	TAT	ACG	CCG	GGT	TCA	TCG	GAA	1968
	Lys	Phe	Trp	Asp	Trp	Leu	Asn	Thr	Lys	Tyr	Thr	Pro	Gly	Ser	Ser	Glu	
					645				650						655		
20	GCC	GTA	GAA	ACG	CAG	GAA	CAT	ATC	GTT	CAG	TAT	TGT	CAG	GCT	CTG	GCA	2016
	Ala	Val	Glu	Thr	Gln	Glu	His	Ile	Val	Gln	Tyr	Cys	Gln	Ala	Leu	Ala	
				660					665					670			
25	CAA	TTG	GAA	ATG	GTT	TAC	CAT	TCC	ACC	GGC	ATC	AAC	GAA	AAC	GCC	TTC	2064
	Gln	Leu	Glu	Met	Val	Tyr	His	Ser	Thr	Gly	Ile	Asn	Glu	Asn	Ala	Phe	
			675					680					685				
30	CGT	CTA	TTT	GTG	ACA	AAA	CCA	GAG	ATG	TTT	GGC	GCT	GCA	ACT	GGA	GCA	2112
	Arg	Leu	Phe	Val	Thr	Lys	Pro	Glu	Met	Phe	Gly	Ala	Ala	Thr	Gly	Ala	
		690					695					700					
35	GCG	CCC	GCG	CAT	GAT	GCC	CTT	TCA	CTG	ATT	ATG	CTG	ACA	CGT	TTT	GCG	2160
	Ala	Pro	Ala	His	Asp	Ala	Leu	Ser	Leu	Ile	Met	Leu	Thr	Arg	Phe	Ala	
	705					710					715					720	
40	GAT	TGG	GTG	AAC	GCA	CTA	GGC	GAA	AAA	GCG	TCC	TCG	GTG	CTA	GCG	GCA	2208
	Asp	Trp	Val	Asn	Ala	Leu	Gly	Glu	Lys	Ala	Ser	Ser	Val	Leu	Ala	Ala	
				725						730					735		
45	TTT	GAA	GCT	AAC	TCG	TTA	ACG	GCA	GAA	CAA	CTG	GCT	GAT	GCC	ATG	AAT	2256
	Phe	Glu	Ala	Asn	Ser	Leu	Thr	Ala	Glu	Gln	Leu	Ala	Asp	Ala	Met	Asn	
				740					745					750			
50	CTT	GAT	GCT	AAT	TTG	CTG	TTG	CAA	GCC	AGT	ATT	CAA	GCA	CAA	AAT	CAT	2304
	Leu	Asp	Ala	Asn	Leu	Leu	Leu	Gln	Ala	Ser	Ile	Gln	Ala	Gln	Asn	His	
			755					760					765				
55	CAA	CAT	CTT	CCC	CCA	GTA	ACT	CCA	GAA	AAT	GCG	TTC	TCC	TGT	TGG	ACA	2352
	Gln	His	Leu	Pro	Pro	Val	Thr	Pro	Glu	Asn	Ala	Phe	Ser	Cys	Trp	Thr	
		770					775					780					
60	TCT	ATC	AAT	ACT	ATC	CTG	CAA	TGG	GTT	AAT	GTC	GCA	CAA	CAA	TTG	AAT	2400
	Ser	Ile	Asn	Thr	Ile	Leu	Gln	Trp	Val	Asn	Val	Ala	Gln	Gln	Leu	Asn	
	785					790					795					800	
65	GTC	GCC	CCA	CAG	GGC	GTT	TCC	GCT	TTG	GTC	GGG	CTG	GAT	TAT	ATT	CAA	2448
	Val	Ala	Pro	Gln	Gly	Val	Ser	Ala	Leu	Val	Gly	Leu	Asp	Tyr	Ile	Gln	
				805						810					815		
70	TCA	ATG	AAA	GAG	ACA	CCG	ACC	TAT	GCC	CAG	TGG	GAA	AAC	GCG	GCA	GGC	2496
	Ser	Met	Lys	Glu	Thr	Pro	Thr	Tyr	Ala	Gln	Trp	Glu	Asn	Ala	Ala	Gly	
				820					825					830			
75	GTA	TTA	ACC	GCC	GGG	TTG	AAT	TCA	CAA	CAG	GCT	AAT	ACA	TTA	CAC	GCT	2544
	Val	Leu	Thr	Ala	Gly	Leu	Asn	Ser	Gln	Gln	Ala	Asn	Thr	Leu	His	Ala	
			835					840					845				
80	TTT	CTG	GAT	GAA	TCT	CGC	AGT	GCC	GCA	TTA	AGC	ACC	TAC	TAT	ATC	CGT	2592
	Phe	Leu	Asp	Glu	Ser	Arg	Ser	Ala	Ala	Leu	Ser	Thr	Tyr	Tyr	Ile	Arg	
		850					855					860					
85	CAA	GTC	GCC	AAG	GCA	GCG	GCG	GCT	ATT	AAA	AGC	CGT	GAT	GAC	TTG	TAT	2640
	Gln	Val	Ala	Lys	Ala	Ala	Ala	Ile	Lys	Ser	Arg	Arg	Asp	Asp	Leu	Tyr	
	865					870					875					880	

	CAA	TAC	TTA	CTG	ATT	GAT	AAT	CAG	GTT	TCT	GCG	GCA	ATA	AAA	ACC	ATC	1688
	Gln	Tyr	Leu	Leu	Ile	Asp	Asn	Gln	Val	Ser	Ala	Ala	Ile	Lys	Thr	Thr	
					385					390					395		
5	CGG	ATC	GCC	GAA	GCC	ATT	GCC	AGT	ATT	CAA	CTG	TAC	CTC	AAC	CGG	GCA	2036
	Arg	Ile	Ala	Glu	Ala	Ile	Ala	Ser	Ile	Gln	Leu	Tyr	Val	Asn	Arg	Ala	
				900					905					910			
10	TTG	GAA	AAT	GTG	GAA	GAA	AAT	GCC	AAT	TCG	GGG	GTT	ATC	AGC	CGC	CAA	1784
	Leu	Glu	Asn	Val	Glu	Glu	Asn	Ala	Asn	Ser	Gly	Val	Ile	Ser	Arg	Gln	
			915					920					925				
15	TTC	TTT	ATC	GAC	TGG	GAC	AAA	TAC	AAT	AAA	GCG	TAC	AGC	ACT	TGG	GCG	2332
	Phe	Phe	Ile	Asp	Trp	Asp	Lys	Tyr	Asn	Lys	Arg	Tyr	Ser	Thr	Trp	Ala	
		930					935					940					
20	GGT	GTT	TCT	CAA	TTA	GTT	TAC	TAC	CCG	GAA	AAC	TAT	ATT	GAT	CCG	ACC	2980
	Gly	Val	Ser	Gln	Leu	Val	Tyr	Tyr	Pro	Glu	Asn	Tyr	Ile	Asp	Pro	Thr	
		945				950					955					960	
	ATG	CGT	ATC	GGA	CAA	ACC	AAA	ATG	ATG	GAC	GCA	TTA	CTG	CAA	TCC	GTC	2928
	Met	Arg	Ile	Gly	Gln	Thr	Lys	Met	Met	Asp	Ala	Leu	Leu	Gln	Ser	Val	
				965						970					975		
25	AGC	CAA	AGC	CAA	TTA	AAC	GCC	GAT	ACC	GTC	GAA	GAT	GCC	TTT	ATG	TCT	2976
	Ser	Gln	Ser	Gln	Leu	Asn	Ala	Asp	Thr	Val	Glu	Asp	Ala	Phe	Met	Ser	
				980					985					990			
30	TAT	CTG	ACA	TCG	TTT	GAA	CAA	GTG	GCT	AAT	CTT	AAA	GTT	ATT	AGC	GCA	3024
	Tyr	Leu	Thr	Ser	Phe	Glu	Gln	Val	Ala	Asn	Leu	Lys	Val	Ile	Ser	Ala	
			995					1000					1005				
35	TAT	CAC	GAT	AAT	ATT	AAT	AAC	GAT	CAA	GGG	CTG	ACC	TAT	TTT	ATC	GGA	3072
	Tyr	His	Asp	Asn	Ile	Asn	Asn	Asp	Gln	Gly	Leu	Thr	Tyr	Phe	Ile	Gly	
		1010					1015					1020					
40	CTC	AGT	GAA	ACT	GAT	GCC	GGT	GAA	TAT	TAT	TGG	GCG	AGT	GTC	GAT	CAC	3120
	Leu	Ser	Glu	Thr	Asp	Ala	Gly	Glu	Tyr	Tyr	Trp	Arg	Ser	Val	Asp	His	
		1025				1030					1035					1040	
	AGT	AAA	TTC	AAC	GAC	GGT	AAA	TTC	GCG	GCT	AAT	GCC	TGG	AGT	GAA	TGG	3168
	Ser	Lys	Phe	Asn	Asp	Gly	Lys	Phe	Ala	Ala	Asn	Ala	Trp	Ser	Glu	Trp	
				1045					1050						1055		
45	CAT	AAA	ATT	GAT	TGT	CCA	ATT	AAC	CCT	TAT	AAA	AGC	ACT	ATC	CGT	CCA	3216
	His	Lys	Ile	Asp	Cys	Pro	Ile	Asn	Pro	Tyr	Lys	Ser	Thr	Ile	Arg	Pro	
				1060					1065					1070			
50	GTG	ATA	TAT	AAA	TCC	GCG	CTG	TAT	CTG	CTC	TGG	TTG	GAA	CAA	AAG	GAG	3264
	Val	Ile	Tyr	Lys	Ser	Arg	Leu	Tyr	Leu	Leu	Trp	Leu	Glu	Gln	Lys	Glu	
			1075				1080						1085				
55	ATC	ACC	AAA	CAG	ACA	GGA	AAT	AGT	AAA	GAT	GCG	TAT	CAA	ACT	GAA	ACG	3312
	Ile	Thr	Lys	Gln	Thr	Gly	Asn	Ser	Lys	Asp	Gly	Tyr	Gln	Thr	Glu	Thr	
		1090					1095					1100					
60	GAT	TAT	CGT	TAT	GAA	CTA	AAA	TTG	GCG	CAT	ATC	GCG	TAT	GAT	GCG	ACT	3360
	Asp	Tyr	Arg	Tyr	Glu	Leu	Lys	Leu	Ala	His	Ile	Arg	Tyr	Asp	Gly	Thr	
		1105				1110					1115					1120	
	TGG	AAT	ACG	CCA	ATC	ACC	TTT	GAT	GTC	AAT	AAA	AAA	ATA	TCC	GAG	CTA	3408
	Trp	Asn	Thr	Pro	Ile	Thr	Phe	Asp	Val	Asn	Lys	Lys	Ile	Ser	Glu	Leu	
					1125				1130						1135		
65	AAA	CTG	GAA	AAA	AAT	AGA	GCG	CCC	GGA	CTC	TAT	TGT	GCC	GGT	TAT	CAA	3456
	Lys	Leu	Glu	Lys	Asn	Arg	Ala	Pro	Gly	Leu	Tyr	Cys	Ala	Gly	Tyr	Gln	
				1140				1145					1150				
70	GGT	GAA	GAT	ACG	TTG	CTG	GTG	ATG	TTT	TAT	AAC	CAA	CAA	GAC	ACA	CTA	3504
	Gly	Glu	Asp	Thr	Leu	Leu	Val	Met	Phe	Tyr	Asn	Gln	Gln	Asp	Thr	Leu	
			1155				1160						1165				

5	GAT AGT TAT AAA AAC GGT TCA ATG CAA GGA CTA TAT ATC TTT GCT GAT 3552	
	Asp Ser Tyr Lys Asn Ala Ser Met Gln Gly Leu Tyr Ile Phe Ala Asp	1170 1175 1180
10	ATG GCA TCC AAA GAT ATG ACC CCA GAA CAG AGC AAT GTT TAT CGG GAT 3600	
	Met Ala Ser Lys Asp Met Thr Pro Glu Gln Ser Asn Val Tyr Arg Asp	1185 1190 1195 1200
15	AAT AGC TAT CAA CAA TTT GAT ACC AAT AAT GTC AGA AGA GTG AAT AAC 3648	
	Asn Ser Tyr Gln Gln Phe Asp Thr Asn Asn Val Arg Arg Val Asn Asn	1205 1210 1215
20	CGC TAT GCA GAG GAT TAT GAG ATT CCT TCC TCG GTA ACT AGC CGT AAA 3696	
	Arg Tyr Ala Glu Asp Tyr Glu Ile Pro Ser Ser Val Ser Ser Arg Lys	1220 1225 1230
25	GAC TAT GGT TGG GGA GAT TAT TAC CTC AGC ATG GTA TAT AAC GGA GAT 3744	
	Asp Tyr Gly Trp Gly Asp Tyr Tyr Leu Ser Met Val Tyr Asn Gly Asp	1235 1240 1245
30	ATT CCA ACT ATC AAT TAC AAA GCC GCA TCA AGT GAT TTA AAA ATC TAT 3792	
	Ile Pro Thr Ile Asn Tyr Lys Ala Ala Ser Ser Asp Leu Lys Ile Tyr	1250 1255 1260
35	ATC TCA CCA AAA TTA AGA ATT ATT CAT AAT GGA TAT GAA GGA CAG AAG 3840	
	Ile Ser Pro Lys Leu Arg Ile Ile His Asn Gly Tyr Glu Gly Gln Lys	1265 1270 1275 1280
40	CGC AAT CAA TGC AAT CTG ATG AAT AAA TAT GGC AAA CTA GGT GAT AAA 3888	
	Arg Asn Gln Cys Asn Leu Met Asn Lys Tyr Gly Lys Leu Gly Asp Lys	1285 1290 1295
45	TTT ATT GTT TAT ACT AGC TTG GGG GTC AAT CCA AAT AAC TCG TCA AAT 3936	
	Phe Ile Val Tyr Thr Ser Leu Gly Val Asn Pro Asn Asn Ser Ser Asn	1300 1305 1310
50	AAG CTC ATG TTT TAC CCC GTC TAT CAA TAT AGC GGA AAC ACC AGT GGA 3984	
	Lys Leu Met Phe Tyr Pro Val Tyr Gln Tyr Ser Gly Asn Thr Ser Gly	1315 1320 1325
55	CTC AAT CAA GGG AGA CTA CTA TTC CAC CGT GAC ACC ACT TAT CCA TCT 4032	
	Leu Asn Gln Gly Arg Leu Leu Phe His Arg Asp Thr Thr Tyr Pro Ser	1330 1335 1340
60	AAA GTA GAA GCT TGG ATT CCT GGA GCA AAA CGT TCT CTA ACC AAC CAA 4080	
	Lys Val Glu Ala Trp Ile Pro Gly Ala Lys Arg Ser Leu Thr Asn Gln	1345 1350 1355 1360
65	AAT GCC GCC ATT GGT GAT GAT TAT GCT ACA GAC TCT CTG AAT AAA CCG 4128	
	Asn Ala Ala Ile Gly Asp Asp Tyr Ala Thr Asp Ser Leu Asn Lys Pro	1365 1370 1375
70	GAT GAT CTT AAG CAA TAT ATC TTT ATG ACT GAC AGT AAA GGG ACT GCT 4176	
	Asp Asp Leu Lys Gln Tyr Ile Phe Met Thr Asp Ser Lys Gly Thr Ala	1380 1385 1390
75	ACT GAT GTC TCA GGC CCA GTA GAG ATT AAT ACT GCA ATT TCT CCA GCA 4224	
	Thr Asp Val Ser Gly Pro Val Glu Ile Asn Thr Ala Ile Ser Pro Ala	1395 1400 1405
80	AAA GTT CAG ATA ATA GTC AAA GCG GGT GGC AAG GAG CAA ACT TTT ACC 4272	
	Lys Val Gln Ile Ile Val Lys Ala Gly Gly Lys Glu Gln Thr Phe Thr	1410 1415 1420
85	GCA GAT AAA GAT GTC TCC ATT CAG CCA TCA CCT AGC TTT GAT GAA ATG 4320	
	Ala Asp Lys Asp Val Ser Ile Gln Pro Ser Pro Ser Phe Asp Glu Met	1425 1430 1435 1440
90	AAT TAT CAA TTT AAT GCC CTT GAA ATA GAC GGT TCT GGT CTG AAT TTT 4368	
	Asn Tyr Gln Phe Asn Ala Leu Glu Ile Asp Gly Ser Gly Leu Asn Phe	

				1445				1450					1455				
				ATT AAC AAC TCA GCC AGT ATT GAT GTT ACT TTT ACC GCA TTT GCG GAG 4416													
5				Ile Asn Asn Ser Ala Ser Ile Asp Val Thr Phe Thr Ala Phe Ala Glu 1460 1465 1470													
				GAT GGC CGC AAA CTG GGT TAT GAA AGT TTC AGT ATT CCT GTT ACC CTC 4464													
				Asp Gly Arg Lys Leu Gly Tyr Glu Ser Phe Ser Ile Pro Val Thr Leu 1475 1480 1485													
10				AAG GTA AGT ACC GAT AAT GCC CTG ACC CTG CAC CAT AAT GAA AAT GGT 4512													
				Lys Val Ser Thr Asp Asn Ala Leu Thr Leu His His Asn Glu Asn Gly 1490 1495 1500													
15				GCG CAA TAT ATG CAA TGG CAA TCC TAT CGT ACC CGC CTG AAT ACT CTA 4560													
				Ala Gln Tyr Met Gln Trp Gln Ser Tyr Arg Thr Arg Leu Asn Thr Leu 1505 1510 1515 1520													
20				TTT GCC CGC CAG TTG GTT GCA CGC GCC ACC ACC GGA ATC GAT ACA ATT 4608													
				Phe Ala Arg Gln Leu Val Ala Arg Ala Thr Thr Gly Ile Asp Thr Ile 1525 1530 1535													
25				CTG AGT ATG GAA ACT CAG AAT ATT CAG GAA CCG CAG TTA GGC AAA GGT 4656													
				Leu Ser Met Glu Thr Gln Asn Ile Gln Glu Pro Gln Leu Gly Lys Gly 1540 1545 1550													
30				TTC TAT GCT ACG TTC GTG ATA CCT CCC TAT AAC CTA TCA ACT CAT GGT 4704													
				Phe Tyr Ala Thr Phe Val Ile Pro Pro Tyr Asn Leu Ser Thr His Gly 1555 1560 1565													
				GAT GAA CGT TGG TTT AAG CTT TAT ATC AAA CAT GTT GTT GAT AAT AAT 4752													
				Asp Glu Arg Trp Phe Lys Leu Tyr Ile Lys His Val Val Asp Asn Asn 1570 1575 1580													
35				TCA CAT ATT ATC TAT TCA GGC CAG CTA ACA GAT ACA AAT ATA AAC ATC 4800													
				Ser His Ile Ile Tyr Ser Gly Gln Leu Thr Asp Thr Asn Ile Asn Ile 1585 1590 1595 1600													
40				ACA TTA TTT ATT CCT CTT GAT GAT GTC CCA TTG AAT CAA GAT TAT CAC 4848													
				Thr Leu Phe Ile Pro Leu Asp Asp Val Pro Leu Asn Gln Asp Tyr His 1605 1610 1615													
45				GCC AAG GTT TAT ATG ACC TTC AAG AAA TCA CCA TCA GAT GGT ACC TGG 4896													
				Ala Lys Val Tyr Met Thr Phe Lys Lys Ser Pro Ser Asp Gly Thr Trp 1620 1625 1630													
50				TGG GGC CCT CAC TTT GTT AGA GAT GAT AAA GGA ATA GTA ACA ATA AAC 4944													
				Trp Gly Pro His Phe Val Arg Asp Asp Lys Gly Ile Val Thr Ile Asn 1635 1640 1645													
				CCT AAA TCC ATT TTG ACC CAT TTT GAG AGC GTC AAT GTC CTG AAT AAT 4992													
				Pro Lys Ser Ile Leu Thr His Phe Glu Ser Val Asn Val Leu Asn Asn 1650 1655 1660													
55				ATT AGT AGC GAA CCA ATG GAT TTC AGC GGC GCT AAC AGC CTC TAT TTC 5040													
				Ile Ser Ser Glu Pro Met Asp Phe Ser Gly Ala Asn Ser Leu Tyr Phe 1665 1670 1675 1680													
60				TGG GAA CTG TTC TAC TAT ACC CCG ATG CTG GTT GCT CAA CGT TTG CTG 5088													
				Trp Glu Leu Phe Tyr Tyr Thr Pro Met Leu Val Ala Gln Arg Leu Leu 1685 1690 1695													
65				CAT GAA CAG AAC TTC GAT GAA GCC AAC CGT TGG CTG AAA TAT GTC TGG 5136													
				His Glu Gln Asn Phe Asp Glu Ala Asn Arg Trp Leu Lys Tyr Val Trp 1700 1705 1710													
				AGT CCA TCC GGT TAT ATT GTC CAC GGC CAG ATT CAG AAC TAC CAG TGG 5184													
				Ser Pro Ser Gly Tyr Ile Val His Gly Gln Ile Gln Asn Tyr Gln Trp 1715 1720 1725													
70				AAC GTC CGC CCG TTA CTG GAA GAC ACC AGT TGG AAC AGT GAT CCT TTG 5232													

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      Asn Val Arg Pro Leu Leu Glu Asp Thr Ser Trp Asn Ser Asp Pro Leu
      1730                1735                1740
5    GAT TCC GTC GAT CCT GAC GCG GTA GCA CAG CAC GAT CCA ATG CAC TAC 5230
      Asp Ser Val Asp Pro Asp Ala Val Ala Gln His Asp Pro Met His Tyr
      1745                1750                1755                1760
10   AAA GTT TCA ACT TTT ATG CGT ACC TTG GAT CTA TTG ATA GCA CGC GGC 5323
      Lys Val Ser Thr Phe Met Arg Thr Leu Asp Leu Leu Ile Ala Arg Gly
      1765                1770                1775
15   GAC CAT GCT TAT CGC CAA CTG GAA CGA GAT ACA CTC AAC GAA GCG AAG 5376
      Asp His Ala Tyr Arg Gln Leu Glu Arg Asp Thr Leu Asn Glu Ala Lys
      1780                1785                1790
20   ATG TGG TAT ATG CAA GCG CTG CAT CTA TTA GGT GAC AAA CCT TAT CTA 5424
      Met Trp Tyr Met Gln Ala Leu His Leu Leu Gly Asp Lys Pro Tyr Leu
      1795                1800                1805
25   CCG CTG AGT ACG ACA TGG AGT GAT CCA CGA CTA GAC AGA GCC GCG GAT 5472
      Pro Leu Ser Thr Thr Trp Ser Asp Pro Arg Leu Asp Arg Ala Ala Asp
      1810                1815                1820
30   ATC ACT ACC CAA AAT GCT CAC GAC AGC GCA ATA GTC GCT CTG CGG CAG 5520
      Ile Thr Thr Gln Asn Ala His Asp Ser Ala Ile Val Ala Leu Arg Gln
      1825                1830                1835                1840
      AAT ATA CCT ACA CCG GCA CCT TTA TCA 5547
      Asn Ile Pro Thr Pro Ala Pro Leu Ser
      1845                1849

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## (2) INFORMATION FOR SEQ ID NO:49:

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35   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 1849 amino acids
      (B) TYPE: amino acids
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

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40   (ii) MOLECULE TYPE: protein

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49 (TcdA<sub>II</sub>):

Features	From	To	Description
Peptide	1	1849	TcdA <sub>II</sub> peptide
Fragment	1	12	S2 N-terminus (SEQ ID NO:13)
Fragment	196	211	(SEQ ID NO:38)
Fragment	466	475	(SEQ ID NO:17)
Fragment	993	1004	(SEQ ID NO:23; 12/13)
Fragment	1297	1312	(SEQ ID NO:18)
Fragment	1390	1409	(SEQ ID NO:39)
Fragment	1532	1554	(SEQ ID NO:21; 19/23)

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55   Leu Ile Gly Tyr Asn Asn Gln Phe Ser Gly Arg Ala Ser Gln Tyr Val
      1           5           10           15
      Ala Pro Gly Thr Val Ser Ser Met Phe Ser Pro Ala Ala Tyr Leu Thr
      20           25           30
60   Glu Leu Tyr Arg Glu Ala Arg Asn Leu His Ala Ser Asp Ser Val Tyr
      35           40           45
      Tyr Leu Asp Thr Arg Arg Pro Asp Leu Lys Ser Met Ala Leu Ser Gln
      50           55           60
65   Gln Asn Met Asp Ile Glu Leu Ser Thr Leu Ser Leu Ser Asn Glu Leu
      65           70           75           80
      Leu Leu Glu Ser Ile Lys Thr Glu Ser Lys Leu Glu Asn Tyr Thr Lys

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	35								30				95			
	Val	Met	Glu	Met	Leu	Ser	Thr	Phe	Arg	Pro	Ser	Gly	Ala	Thr	Pro	Tyr
				100					105					110		
5	His	Asp	Ala	Tyr	Glu	Asn	Val	Arg	Glu	Val	Ile	Gln	Leu	Gln	Asp	Pro
			115					120					125			
10	Gly	Leu	Glu	Gln	Leu	Asn	Ala	Ser	Pro	Ala	Ile	Ala	Gly	Leu	Met	His
		130					135					140				
	Gln	Ala	Ser	Leu	Leu	Gly	Ile	Asn	Ala	Ser	Ile	Ser	Pro	Glu	Leu	Phe
	145					150					155					160
15	Asn	Ile	Leu	Thr	Glu	Glu	Ile	Thr	Glu	Gly	Asn	Ala	Glu	Glu	Leu	Tyr
				165						170					175	
	Lys	Lys	Asn	Phe	Gly	Asn	Ile	Glu	Pro	Ala	Ser	Leu	Ala	Met	Pro	Glu
				180					185					190		
20	Tyr	Leu	Lys	Arg	Tyr	Tyr	Asn	Leu	Ser	Asp	Glu	Glu	Leu	Ser	Gln	Phe
			195					200					205			
	Ile	Gly	Lys	Ala	Ser	Asn	Phe	Gly	Gln	Gln	Glu	Tyr	Ser	Asn	Asn	Gln
25		210					215					220				
	Leu	Ile	Thr	Pro	Val	Val	Asn	Ser	Ser	Asp	Gly	Thr	Val	Lys	Val	Tyr
	225					230					235					240
30	Arg	Ile	Thr	Arg	Glu	Tyr	Thr	Thr	Asn	Ala	Tyr	Gln	Met	Asp	Val	Glu
				245						250					255	
	Leu	Phe	Pro	Phe	Gly	Gly	Glu	Asn	Tyr	Arg	Leu	Asp	Tyr	Lys	Phe	Lys
				260					265					270		
35	Asn	Phe	Tyr	Asn	Ala	Ser	Tyr	Leu	Ser	Ile	Lys	Leu	Asn	Asp	Lys	Arg
			275					280					285			
	Glu	Leu	Val	Arg	Thr	Glu	Gly	Ala	Pro	Gln	Val	Asn	Ile	Glu	Tyr	Ser
40		290					295					300				
	Ala	Asn	Ile	Thr	Leu	Asn	Thr	Ala	Asp	Ile	Ser	Gln	Pro	Phe	Glu	Ile
	305					310					315					320
45	Gly	Leu	Thr	Arg	Val	Leu	Pro	Ser	Gly	Ser	Trp	Ala	Tyr	Ala	Ala	Ala
				325						330					335	
	Lys	Phe	Thr	Val	Glu	Glu	Tyr	Asn	Gln	Tyr	Ser	Phe	Leu	Leu	Lys	Leu
				340					345					350		
50	Asn	Lys	Ala	Ile	Arg	Leu	Ser	Arg	Ala	Thr	Glu	Leu	Ser	Pro	Thr	Ile
			355					360					365			
	Leu	Glu	Gly	Ile	Val	Arg	Ser	Val	Asn	Leu	Gln	Leu	Asp	Ile	Asn	Thr
55		370					375					380				
	Asp	Val	Leu	Gly	Lys	Val	Phe	Leu	Thr	Lys	Tyr	Tyr	Met	Gln	Arg	Tyr
	385					390					395					400
60	Ala	Ile	His	Ala	Glu	Thr	Ala	Leu	Ile	Leu	Cys	Asn	Ala	Pro	Ile	Ser
				405						410					415	
	Gln	Arg	Ser	Tyr	Asp	Asn	Gln	Pro	Ser	Gln	Phe	Asp	Arg	Leu	Phe	Asn
				420					425					430		
65	Thr	Pro	Leu	Leu	Asn	Gly	Gln	Tyr	Phe	Ser	Thr	Gly	Asp	Glu	Glu	Ile
			435					440					445			
	Asp	Leu	Asn	Ser	Gly	Ser	Thr	Gly	Asp	Trp	Arg	Lys	Thr	Ile	Leu	Lys
70		450					455					460				



	Arg	Ala	Phe	Asn	Ile	Asp	Asp	Val	Ser	Leu	Phe	Arg	Leu	Leu	Lys	Ile	
	465					470					475					480	
5	Thr	Asp	His	Asp	Asn	Lys	Asp	Gly	Lys	Ile	Lys	Asn	Asn	Leu	Lys	Asn	
					485					490					495		
	Leu	Ser	Asn	Leu	Tyr	Ile	Gly	Lys	Leu	Leu	Ala	Asp	Ile	His	Gln	Leu	
				500					505					510			
10	Thr	Ile	Asp	Glu	Leu	Asp	Leu	Leu	Leu	Ile	Ala	Val	Gly	Glu	Gly	Lys	
			515					520					525				
	Thr	Asn	Leu	Ser	Ala	Ile	Ser	Asp	Lys	Gln	Leu	Ala	Thr	Leu	Ile	Arg	
			530				535					540					
15	Lys	Leu	Asn	Thr	Ile	Thr	Ser	Trp	Leu	His	Thr	Gln	Lys	Trp	Ser	Val	
	545					550					555					560	
	Phe	Gln	Leu	Phe	Ile	Met	Thr	Ser	Thr	Ser	Tyr	Asn	Lys	Thr	Leu	Thr	
20					565					570					575		
	Pro	Glu	Ile	Lys	Asn	Leu	Leu	Asp	Thr	Val	Tyr	His	Gly	Leu	Gln	Gly	
				580					585					590			
25	Phe	Asp	Lys	Asp	Lys	Ala	Asp	Leu	Leu	His	Val	Met	Ala	Pro	Tyr	Ile	
			595					600					605				
	Ala	Ala	Thr	Leu	Gln	Leu	Ser	Ser	Glu	Asn	Val	Ala	His	Ser	Val	Leu	
			610				615					620					
30	Leu	Trp	Ala	Asp	Lys	Leu	Gln	Pro	Gly	Asp	Gly	Ala	Met	Thr	Ala	Glu	
	625					630					635					640	
	Lys	Phe	Trp	Asp	Trp	Leu	Asn	Thr	Lys	Tyr	Thr	Pro	Gly	Ser	Ser	Glu	
35					645					650					655		
	Ala	Val	Glu	Thr	Gln	Glu	His	Ile	Val	Gln	Tyr	Cys	Gln	Ala	Leu	Ala	
				660					665					670			
40	Gln	Leu	Glu	Met	Val	Tyr	His	Ser	Thr	Gly	Ile	Asn	Glu	Asn	Ala	Phe	
			675					680					685				
	Arg	Leu	Phe	Val	Thr	Lys	Pro	Glu	Met	Phe	Gly	Ala	Ala	Thr	Gly	Ala	
			690				695					700					
45	Ala	Pro	Ala	His	Asp	Ala	Leu	Ser	Leu	Ile	Met	Leu	Thr	Arg	Phe	Ala	
	705					710					715					720	
	Asp	Trp	Val	Asn	Ala	Leu	Gly	Glu	Lys	Ala	Ser	Ser	Val	Leu	Ala	Ala	
					725					730					735		
50	Phe	Glu	Ala	Asn	Ser	Leu	Thr	Ala	Glu	Gln	Leu	Ala	Asp	Ala	Met	Asn	
				740					745					750			
	Leu	Asp	Ala	Asn	Leu	Leu	Leu	Gln	Ala	Ser	Ile	Gln	Ala	Gln	Asn	His	
55			755					760					765				
	Gln	His	Leu	Pro	Pro	Val	Thr	Pro	Glu	Asn	Ala	Phe	Ser	Cys	Trp	Thr	
			770				775					780					
60	Ser	Ile	Asn	Thr	Ile	Leu	Gln	Trp	Val	Asn	Val	Ala	Gln	Gln	Leu	Asn	
	785					790					795					800	
	Val	Ala	Pro	Gln	Gly	Val	Ser	Ala	Leu	Val	Gly	Leu	Asp	Tyr	Ile	Gln	
					805					810					815		
65	Ser	Met	Lys	Glu	Thr	Pro	Thr	Tyr	Ala	Gln	Trp	Glu	Asn	Ala	Ala	Gly	
				820					825					830			
	Val	Leu	Thr	Ala	Gly	Leu	Asn	Ser	Gln	Gln	Ala	Asn	Thr	Leu	His	Ala	
70				835				840					845				

	Phe	Leu	Asp	Glu	Ser	Arg	Ser	Ala	Ala	Leu	Ser	Thr	Tyr	Tyr	Ile	Arg	
	350						355					860					
5	Gln	Val	Ala	Lys	Ala	Ala	Ala	Ile	Lys	Ser	Arg	Asp	Asp	Leu	Tyr		
	365					370				375					880		
	Gln	Tyr	Leu	Leu	Ile	Asp	Asn	Gln	Val	Ser	Ala	Ala	Ile	Lys	Thr	Thr	
					885					890					895		
10	Arg	Ile	Ala	Glu	Ala	Ile	Ala	Ser	Ile	Gln	Leu	Tyr	Val	Asn	Arg	Ala	
				900					905					910			
	Leu	Glu	Asn	Val	Glu	Glu	Asn	Ala	Asn	Ser	Gly	Val	Ile	Ser	Arg	Gln	
15			915					920					925				
	Phe	Phe	Ile	Asp	Trp	Asp	Lys	Tyr	Asn	Lys	Arg	Tyr	Ser	Thr	Trp	Ala	
	930						935					940					
20	Gly	Val	Ser	Gln	Leu	Val	Tyr	Tyr	Pro	Glu	Asn	Tyr	Ile	Asp	Pro	Thr	
	945					950					955					960	
	Met	Arg	Ile	Gly	Gln	Thr	Lys	Met	Met	Asp	Ala	Leu	Leu	Gln	Ser	Val	
					965					970					975		
25	Ser	Gln	Ser	Gln	Leu	Asn	Ala	Asp	Thr	Val	Glu	Asp	Ala	Phe	Met	Ser	
				980					985					990			
	Tyr	Leu	Thr	Ser	Phe	Glu	Gln	Val	Ala	Asn	Leu	Lys	Val	Ile	Ser	Ala	
30				995				1000					1005				
	Tyr	His	Asp	Asn	Ile	Asn	Asn	Asp	Gln	Gly	Leu	Thr	Tyr	Phe	Ile	Gly	
	1010					1015						1020					
35	Leu	Ser	Glu	Thr	Asp	Ala	Gly	Glu	Tyr	Tyr	Trp	Arg	Ser	Val	Asp	His	
	1025					1030					1035					1040	
	Ser	Lys	Phe	Asn	Asp	Gly	Lys	Phe	Ala	Ala	Asn	Ala	Trp	Ser	Glu	Trp	
				1045					1050						1055		
40	His	Lys	Ile	Asp	Cys	Pro	Ile	Asn	Pro	Tyr	Lys	Ser	Thr	Ile	Arg	Pro	
				1060				1065						1070			
	Val	Ile	Tyr	Lys	Ser	Arg	Leu	Tyr	Leu	Leu	Trp	Leu	Glu	Gln	Lys	Glu	
45			1075				1080						1085				
	Ile	Thr	Lys	Gln	Thr	Gly	Asn	Ser	Lys	Asp	Gly	Tyr	Gln	Thr	Glu	Thr	
	1090					1095						1100					
50	Asp	Tyr	Arg	Tyr	Glu	Leu	Lys	Leu	Ala	His	Ile	Arg	Tyr	Asp	Gly	Thr	
	1105					1110				1115						1120	
	Trp	Asn	Thr	Pro	Ile	Thr	Phe	Asp	Val	Asn	Lys	Lys	Ile	Ser	Glu	Leu	
				1125					1130						1135		
55	Lys	Leu	Glu	Lys	Asn	Arg	Ala	Pro	Gly	Leu	Tyr	Cys	Ala	Gly	Tyr	Gln	
				1140				1145						1150			
	Gly	Glu	Asp	Thr	Leu	Leu	Val	Met	Phe	Tyr	Asn	Gln	Gln	Asp	Thr	Leu	
60			1155				1160					1165					
	Asp	Ser	Tyr	Lys	Asn	Ala	Ser	Met	Gln	Gly	Leu	Tyr	Ile	Phe	Ala	Asp	
	1170					1175						1180					
65	Met	Ala	Ser	Lys	Asp	Met	Thr	Pro	Glu	Gln	Ser	Asn	Val	Tyr	Arg	Asp	
	1185					1190					1195					1200	
	Asn	Ser	Tyr	Gln	Gln	Phe	Asp	Thr	Asn	Asn	Val	Arg	Arg	Val	Asn	Asn	
				1205					1210						1215		
70	Arg	Tyr	Ala	Glu	Asp	Tyr	Glu	Ile	Pro	Ser	Ser	Val	Ser	Ser	Arg	Lys	
				1220					1225						1230		

	Asp Tyr Gly Trp Gly Asp Tyr Tyr Leu Ser Met Val Tyr Asn Gly Asp	1235	1240	1245
5	Ile Pro Thr Ile Asn Tyr Lys Ala Ala Ser Ser Asp Leu Lys Ile Tyr	1250	1255	1260
	Ile Ser Pro Lys Leu Arg Ile Ile His Asn Gly Tyr Glu Gly Gln Lys	1265	1270	1275
10	Arg Asn Gln Cys Asn Leu Met Asn Lys Tyr Gly Lys Leu Gly Asp Lys	1285	1290	1295
	Phe Ile Val Tyr Thr Ser Leu Gly Val Asn Pro Asn Asn Ser Ser Asn	1300	1305	1310
15	Lys Leu Met Phe Tyr Pro Val Tyr Gln Tyr Ser Gly Asn Thr Ser Gly	1315	1320	1325
	Leu Asn Gln Gly Arg Leu Leu Phe His Arg Asp Thr Thr Tyr Pro Ser	1330	1335	1340
	Lys Val Glu Ala Trp Ile Pro Gly Ala Lys Arg Ser Leu Thr Asn Gln	1345	1350	1355
25	Asn Ala Ala Ile Gly Asp Asp Tyr Ala Thr Asp Ser Leu Asn Lys Pro	1365	1370	1375
	Asp Asp Leu Lys Gln Tyr Ile Phe Met Thr Asp Ser Lys Gly Thr Ala	1380	1385	1390
30	Thr Asp Val Ser Gly Pro Val Glu Ile Asn Thr Ala Ile Ser Pro Ala	1395	1400	1405
	Lys Val Gln Ile Ile Val Lys Ala Gly Gly Lys Glu Gln Thr Phe Thr	1410	1415	1420
	Ala Asp Lys Asp Val Ser Ile Gln Pro Ser Pro Ser Phe Asp Glu Met	1425	1430	1435
40	Asn Tyr Gln Phe Asn Ala Leu Glu Ile Asp Gly Ser Gly Leu Asn Phe	1445	1450	1455
	Ile Asn Asn Ser Ala Ser Ile Asp Val Thr Phe Thr Ala Phe Ala Glu	1460	1465	1470
45	Asp Gly Arg Lys Leu Gly Tyr Glu Ser Phe Ser Ile Pro Val Thr Leu	1475	1480	1485
	Lys Val Ser Thr Asp Asn Ala Leu Thr Leu His His Asn Glu Asn Gly	1490	1495	1500
	Ala Gln Tyr Met Gln Trp Gln Ser Tyr Arg Thr Arg Leu Asn Thr Leu	1505	1510	1515
55	Phe Ala Arg Gln Leu Val Ala Arg Ala Thr Thr Gly Ile Asp Thr Ile	1525	1530	1535
	Leu Ser Met Glu Thr Gln Asn Ile Gln Glu Pro Gln Leu Gly Lys Gly	1540	1545	1550
60	Phe Tyr Ala Thr Phe Val Ile Pro Pro Tyr Asn Leu Ser Thr His Gly	1555	1560	1565
	Asp Glu Arg Trp Phe Lys Leu Tyr Ile Lys His Val Val Asp Asn Asn	1570	1575	1580
	Ser His Ile Ile Tyr Ser Gly Gln Leu Thr Asp Thr Asn Ile Asn Ile	1585	1590	1595
70	Thr Leu Phe Ile Pro Leu Asp Asp Val Pro Leu Asn Gln Asp Tyr His			

1605 1610 1615

Ala Lys Val Tyr Met Thr Phe Lys Lys Ser Pro Ser Asp Gly Thr Trp  
1620 1625 1630

5 Trp Gly Pro His Phe Val Arg Asp Asp Lys Gly Ile Val Thr Ile Asn  
1635 1640 1645

10 Pro Lys Ser Ile Leu Thr His Phe Glu Ser Val Asn Val Leu Asn Asn  
1650 1655 1660

Ile Ser Ser Glu Pro Met Asp Phe Ser Gly Ala Asn Ser Leu Tyr Phe  
1665 1670 1675 1680

15 Trp Glu Leu Phe Tyr Tyr Thr Pro Met Leu Val Ala Gln Arg Leu Leu  
1685 1690 1695

His Glu Gln Asn Phe Asp Glu Ala Asn Arg Trp Leu Lys Tyr Val Trp  
1700 1705 1710

20 Ser Pro Ser Gly Tyr Ile Val His Gly Gln Ile Gln Asn Tyr Gln Trp  
1715 1720 1725

25 Asn Val Arg Pro Leu Leu Glu Asp Thr Ser Trp Asn Ser Asp Pro Leu  
1730 1735 1740

Asp Ser Val Asp Pro Asp Ala Val Ala Gln His Asp Pro Met His Tyr  
1745 1750 1755 1760

30 Lys Val Ser Thr Phe Met Arg Thr Leu Asp Leu Leu Ile Ala Arg Gly  
1765 1770 1775

Asp His Ala Tyr Arg Gln Leu Glu Arg Asp Thr Leu Asn Glu Ala Lys  
1780 1785 1790

35 Met Trp Tyr Met Gln Ala Leu His Leu Leu Gly Asp Lys Pro Tyr Leu  
1795 1800 1805

40 Pro Leu Ser Thr Thr Trp Ser Asp Pro Arg Leu Asp Arg Ala Ala Asp  
1810 1815 1820

Ile Thr Thr Gln Asn Ala His Asp Ser Ala Ile Val Ala Leu Arg Gln  
1825 1830 1835 1840

45 Asn Ile Pro Thr Pro Ala Pro Leu Ser  
1845 1849

(2) INFORMATION FOR SEQ ID NO:50:

50 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 1740 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

55 (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50 (TcdA<sub>iii</sub> coding region):

60 TTG CGC AGC GCT AAT ACC CTG ACT GAT CTC TTC CTG CCG CAA ATC AAT 48  
Leu Arg Ser Ala Asn Thr Leu Thr Asp Leu Phe Leu Pro Gln Ile Asn  
1 5 10 15

65 GAA GTG ATG ATG AAT TAC TGG CAG ACA TTA GCT CAG AGA GTA TAC AAT 96  
Glu Val Met Met Asn Tyr Trp Gln Thr Leu Ala Gln Arg Val Tyr Asn  
20 25 30

CTG CGT CAT AAC CTC TCT ATC GAC GGC CAG CCG TTA TAT CTG CCA ATC 144

	Leu	Arg	His	Asn	Leu	Ser	Ile	Asp	Gly	Gln	Pro	Leu	Tyr	Leu	Pro	Ile	
			35					40					45				
5	TAT	GCC	ACA	CCG	GCC	GAT	CCG	AAA	GCG	TTA	CTC	AGC	GCC	GCC	GTT	GCC	192
	Tyr	Ala	Thr	Pro	Ala	Asp	Pro	Lys	Ala	Leu	Leu	Ser	Ala	Ala	Val	Ala	
		50					55					50					
10	ACT	TCT	CAA	GGT	GGA	GGC	AAG	CTA	CCG	GAA	TCA	TTT	ATG	TCC	CTG	TGG	240
	Thr	Ser	Gln	Gly	Gly	Gly	Lys	Leu	Pro	Glu	Ser	Phe	Met	Ser	Leu	Trp	
	65					70					75					80	
15	CGT	TTC	CCG	CAC	ATG	CTG	GAA	AAT	GCG	CGC	GCC	ATG	GTT	AGC	CAG	CTC	288
	Arg	Phe	Pro	His	Met	Leu	Glu	Asn	Ala	Arg	Gly	Met	Val	Ser	Gln	Leu	
					85					90					95		
	ACC	CAG	TTC	GGC	TCC	ACG	TTA	CAA	AAT	ATT	ATC	GAA	CGT	CAG	GAC	GCG	336
	Thr	Gln	Phe	Gly	Ser	Thr	Leu	Gln	Asn	Ile	Ile	Glu	Arg	Gln	Asp	Ala	
				100					105					110			
20	GAA	GCG	CTC	AAT	GCG	TTA	TTA	CAA	AAT	CAG	GCC	GCC	GAG	CTG	ATA	TTG	384
	Glu	Ala	Leu	Asn	Ala	Leu	Leu	Gln	Asn	Gln	Ala	Ala	Glu	Leu	Ile	Leu	
			115					120					125				
25	ACT	AAC	CTG	AGC	ATT	CAG	GAC	AAA	ACC	ATT	GAA	GAA	TTG	GAT	GCC	GAG	432
	Thr	Asn	Leu	Ser	Ile	Gln	Asp	Lys	Thr	Ile	Glu	Glu	Leu	Asp	Ala	Glu	
		130					135					140					
30	AAA	ACG	GTG	TTG	GAA	AAA	TCC	AAA	GCG	GGA	GCA	CAA	TCG	CGC	TTT	GAT	480
	Lys	Thr	Val	Leu	Glu	Lys	Ser	Lys	Ala	Gly	Ala	Gln	Ser	Arg	Phe	Asp	
	145					150					155					160	
35	AGC	TAC	GGC	AAA	CTG	TAC	GAT	GAG	AAT	ATC	AAC	GCC	GGT	GAA	AAC	CAA	528
	Ser	Tyr	Gly	Lys	Leu	Tyr	Asp	Glu	Asn	Ile	Asn	Ala	Gly	Glu	Asn	Gln	
					165					170					175		
	GCC	ATG	ACG	CTA	CGA	GCG	TCC	GCC	GCC	GGG	CTT	ACC	ACG	GCA	GTT	CAG	576
	Ala	Met	Thr	Leu	Arg	Ala	Ser	Ala	Ala	Gly	Leu	Thr	Thr	Ala	Val	Gln	
				180				185						190			
40	GCA	TCC	CGT	CTG	GCC	GGT	GCG	GCG	GCT	GAT	CTG	GTG	CCT	AAC	ATC	TTC	624
	Ala	Ser	Arg	Leu	Ala	Gly	Ala	Ala	Ala	Asp	Leu	Val	Pro	Asn	Ile	Phe	
			195					200					205				
45	GGC	TTT	GCC	GGT	GGC	GGC	AGC	CGT	TGG	GGG	GCT	ATC	GCT	GAG	GCG	ACA	672
	Gly	Phe	Ala	Gly	Gly	Gly	Ser	Arg	Trp	Gly	Ala	Ile	Ala	Glu	Ala	Thr	
		210					215					220					
50	GGT	TAT	GTG	ATG	GAA	TTC	TCC	GCG	AAT	GTT	ATG	AAC	ACC	GAA	GCG	GAT	720
	Gly	Tyr	Val	Met	Glu	Phe	Ser	Ala	Asn	Val	Met	Asn	Thr	Glu	Ala	Asp	
	225					230					235					240	
55	AAA	ATT	AGC	CAA	TCT	GAA	ACC	TAC	CGT	CGT	CGC	CGT	CAG	GAG	TGG	GAG	768
	Lys	Ile	Ser	Gln	Ser	Glu	Thr	Tyr	Arg	Arg	Arg	Arg	Gln	Glu	Trp	Glu	
					245					250					255		
	ATC	CAG	CGG	AAT	AAT	GCC	GAA	GCG	GAA	TTG	AAG	CAA	ATC	GAT	GCT	CAG	816
	Ile	Gln	Arg	Asn	Asn	Ala	Glu	Ala	Glu	Leu	Lys	Gln	Ile	Asp	Ala	Gln	
				260				265						270			
60	CTC	AAA	TCA	CTC	GCT	GTA	CGC	CGC	GAA	GCC	GCC	GTA	TTG	CAG	AAA	ACC	864
	Leu	Lys	Ser	Leu	Ala	Val	Arg	Arg	Glu	Ala	Ala	Val	Leu	Gln	Lys	Thr	
			275					280					285				
65	AGT	CTG	AAA	ACC	CAA	CAA	GAA	CAG	ACC	CAA	TCT	CAA	TTG	GCC	TTC	CTG	912
	Ser	Leu	Lys	Thr	Gln	Gln	Glu	Gln	Thr	Gln	Ser	Gln	Leu	Ala	Phe	Leu	
		290					295					300					
70	CAA	CGT	AAG	TTC	AGC	AAT	CAG	GCG	TTA	TAC	AAC	TGG	CTG	CGT	GGT	CGA	960
	Gln	Arg	Lys	Phe	Ser	Asn	Gln	Ala	Leu	Tyr	Asn	Trp	Leu	Arg	Gly	Arg	
	305					310					315					320	

	CTG GCG GCG ATT TAC TTC CAG TTC TAC GAT TTG GCG JTC GCG CGT TGC 1008
	Leu Ala Ala Ile Tyr Phe Gln Phe Tyr Asp Leu Ala Val Ala Arg Cys 335
5	CTG ATG GCA GAA CAA GCT TAC CGT TGG GAA CTC AAT GAT GAC TCT GCC 1056
	Leu Met Ala Glu Gln Ala Tyr Arg Trp Glu Leu Asn Asp Asp Ser Ala 350
10	CGC TTC ATT AAA CCG GGC GCC TGG CAG GGA ACC TAT GCC GGT CTG CTT 1104
	Arg Phe Ile Lys Pro Gly Ala Trp Gln Gly Thr Tyr Ala Gly Leu Leu 365
15	GCA GGT GAA ACC TTG ATG CTG AGT CTG GCA CAA ATG GAA GAC GCT CAT 1152
	Ala Gly Glu Thr Leu Met Leu Ser Leu Ala Gln Met Glu Asp Ala His 380
20	CTG AAA CGC GAT AAA CGC GCA TTA GAG GTT GAA CGC ACA GTA TCG CTG 1200
	Leu Lys Arg Asp Lys Arg Ala Leu Glu Val Glu Arg Thr Val Ser Leu 400
	GCC GAA GTT TAT GCA GGA TTA CCA AAA GAT AAC GGT CCA TTT TCC CTG 1248
	Ala Glu Val Tyr Ala Gly Leu Pro Lys Asp Asn Gly Pro Phe Ser Leu 415
25	GCT CAG GAA ATT GAC AAG CTG GTG AGT CAA GGT TCA GGC AGT GCC GGC 1296
	Ala Gln Glu Ile Asp Lys Leu Val Ser Gln Gly Ser Gly Ser Ala Gly 430
30	AGT GGT AAT AAT AAT TTG GCG TTC GGC GCC GGC ACG GAC ACT AAA ACC 1344
	Ser Gly Asn Asn Asn Leu Ala Phe Gly Ala Gly Thr Asp Thr Lys Thr 445
35	TCT TTG CAG GCA TCA GTT TCA TTC GCT GAT TTG AAA ATT CGT GAA GAT 1392
	Ser Leu Gln Ala Ser Val Ser Phe Ala Asp Leu Lys Ile Arg Glu Asp 460
40	TAC CCG GCA TCG CTT GGC AAA ATT CGA CGT ATC AAA CAG ATC AGC GTC 1440
	Tyr Pro Ala Ser Leu Gly Lys Ile Arg Arg Ile Lys Gln Ile Ser Val 480
	ACT TTG CCC GCG CTA CTG GGA CCG TAT CAG GAT GTA CAG GCA ATA TTG 1488
	Thr Leu Pro Ala Leu Leu Gly Pro Tyr Gln Asp Val Gln Ala Ile Leu 495
45	TCT TAC GGC GAT AAA GCC GGA TTA GCT AAC GGC TGT GAA GCG CTG GCA 1536
	Ser Tyr Gly Asp Lys Ala Gly Leu Ala Asn Gly Cys Glu Ala Leu Ala 510
50	GTT TCT CAC GGT ATG AAT GAC AGC GGC CAA TTC CAG CTC GAT TTC AAC 1584
	Val Ser His Gly Met Asn Asp Ser Gly Gln Phe Gln Leu Asp Phe Asn 525
55	GAT GGC AAA TTC CTG CCA TTC GAA GGC ATC GCC ATT GAT CAA GGC ACG 1632
	Asp Gly Lys Phe Leu Pro Phe Glu Gly Ile Ala Ile Asp Gln Gly Thr 540
60	CTG ACA CTG AGC TTC CCA AAT GCA TCT ATG CCG GAG AAA GGT AAA CAA 1680
	Leu Thr Leu Ser Phe Pro Asn Ala Ser Met Pro Glu Lys Gly Lys Gln 560
	GCC ACT ATG TTA AAA ACC CTG AAC GAT ATC ATT TTG CAT ATT CGC TAC 1728
	Ala Thr Met Leu Lys Thr Leu Asn Asp Ile Ile Leu His Ile Arg Tyr 575
65	ACC ATT AAA TAA 1740
	Thr Ile Lys ... 579

70 (2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 579 amino acids  
 (B) TYPE: amino acids  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51 (TcdAiii):

10 Leu Arg Ser Ala Asn Thr Leu Thr Asp Leu Phe Leu Pro Gln Ile Asn  
 1 5 10 15  
 15 Glu Val Met Met Asn Tyr Trp Gln Thr Leu Ala Gln Arg Val Tyr Asn  
 20 25 30  
 Leu Arg His Asn Leu Ser Ile Asp Gly Gln Pro Leu Tyr Leu Pro Ile  
 35 40 45  
 20 Tyr Ala Thr Pro Ala Asp Pro Lys Ala Leu Leu Ser Ala Ala Val Ala  
 50 55 60  
 Thr Ser Gln Gly Gly Gly Lys Leu Pro Glu Ser Phe Met Ser Leu Trp  
 65 70 75 80  
 25 Arg Phe Pro His Met Leu Glu Asn Ala Arg Gly Met Val Ser Gln Leu  
 85 90 95  
 Thr Gln Phe Gly Ser Thr Leu Gln Asn Ile Ile Glu Arg Gln Asp Ala  
 100 105 110  
 Glu Ala Leu Asn Ala Leu Leu Gln Asn Gln Ala Ala Glu Leu Ile Leu  
 115 120 125  
 35 Thr Asn Leu Ser Ile Gln Asp Lys Thr Ile Glu Glu Leu Asp Ala Glu  
 130 135 140  
 Lys Thr Val Leu Glu Lys Ser Lys Ala Gly Ala Gln Ser Arg Phe Asp  
 145 150 155 160  
 40 Ser Tyr Gly Lys Leu Tyr Asp Glu Asn Ile Asn Ala Gly Glu Asn Gln  
 165 170 175  
 Ala Met Thr Leu Arg Ala Ser Ala Ala Gly Leu Thr Thr Ala Val Gln  
 180 185 190  
 Ala Ser Arg Leu Ala Gly Ala Ala Ala Asp Leu Val Pro Asn Ile Phe  
 195 200 205  
 50 Gly Phe Ala Gly Gly Gly Ser Arg Trp Gly Ala Ile Ala Glu Ala Thr  
 210 215 220  
 Gly Tyr Val Met Glu Phe Ser Ala Asn Val Met Asn Thr Glu Ala Asp  
 225 230 235 240  
 55 Lys Ile Ser Gln Ser Glu Thr Tyr Arg Arg Arg Gln Glu Trp Glu  
 245 250 255  
 Ile Gln Arg Asn Asn Ala Glu Ala Glu Leu Lys Gln Ile Asp Ala Gln  
 260 265 270  
 Leu Lys Ser Leu Ala Val Arg Arg Glu Ala Ala Val Leu Gln Lys Thr  
 275 280 285  
 65 Ser Leu Lys Thr Gln Gln Glu Gln Thr Gln Ser Gln Leu Ala Phe Leu  
 290 295 300  
 Gln Arg Lys Phe Ser Asn Gln Ala Leu Tyr Asn Trp Leu Arg Gly Arg  
 305 310 315 320  
 70

Leu Ala Ala Ile Tyr Phe Gln Phe Tyr Asp Leu Ala Val Ala Arg Cys  
 325 330 335  
 5 Leu Met Ala Glu Gln Ala Tyr Arg Trp Glu Leu Asn Asp Asp Ser Ala  
 340 345 350  
 Arg Phe Ile Lys Pro Gly Ala Trp Gln Gly Thr Tyr Ala Gly Leu Leu  
 355 360 365  
 10 Ala Gly Glu Thr Leu Met Leu Ser Leu Ala Gln Met Glu Asp Ala His  
 370 375 380  
 Leu Lys Arg Asp Lys Arg Ala Leu Glu Val Glu Arg Thr Val Ser Leu  
 385 390 395 400  
 15 Ala Glu Val Tyr Ala Gly Leu Pro Lys Asp Asn Gly Pro Phe Ser Leu  
 405 410 415  
 Ala Gln Glu Ile Asp Lys Leu Val Ser Gln Gly Ser Gly Ser Ala Gly  
 420 425 430  
 Ser Gly Asn Asn Asn Leu Ala Phe Gly Ala Gly Thr Asp Thr Lys Thr  
 435 440 445  
 25 Ser Leu Gln Ala Ser Val Ser Phe Ala Asp Leu Lys Ile Arg Glu Asp  
 450 455 460  
 Tyr Pro Ala Ser Leu Gly Lys Ile Arg Arg Ile Lys Gln Ile Ser Val  
 465 470 475 480  
 30 Thr Leu Pro Ala Leu Leu Gly Pro Tyr Gln Asp Val Gln Ala Ile Leu  
 485 490 495  
 Ser Tyr Gly Asp Lys Ala Gly Leu Ala Asn Gly Cys Glu Ala Leu Ala  
 500 505 510  
 35 Val Ser His Gly Met Asn Asp Ser Gly Gln Phe Gln Leu Asp Phe Asn  
 515 520 525  
 40 Asp Gly Lys Phe Leu Pro Phe Glu Gly Ile Ala Ile Asp Gln Gly Thr  
 530 535 540  
 Leu Thr Leu Ser Phe Pro Asn Ala Ser Met Pro Glu Lys Gly Lys Gln  
 545 550 555 560  
 45 Ala Thr Met Leu Lys Thr Leu Asn Asp Ile Ile Leu His Ile Arg Tyr  
 565 570 575  
 50 Thr Ile Lys ...  
 579

## (2) INFORMATION FOR SEQ ID NO:52:

- 55 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 5532 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear  
 60 (ii) MOLECULE TYPE: DNA (genomic)  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:52 (TcdA<sub>III</sub> coding region):  
 65 TTT ATA CAA GGT TAT AGT GAT CTG TTT GGT AAT CGT GCT GAT AAC TAT 48  
 Phe Ile Gln Gly Tyr Ser Asp Leu Phe Gly Asn Arg Ala Asp Asn Tyr  
 1 5 10 15  
 GCC GCG CCG GGC TCG GTT GCA TCG ATG TTC TCA CCG GCG GCT TAT TTG 96



	Ala	Ala	Pro	Gly	Ser	Val	Ala	Ser	Met	Phe	Ser	Pro	Ala	Ala	Tyr	Leu	
				20					25					30			
5	ACG	GAA	TTG	TAC	CGT	GAA	GCC	AAA	AAC	TTG	CAT	GAC	AGC	AGC	TCA	ATT	144
	Thr	Glu	Leu	Tyr	Arg	Glu	Ala	Lys	Asn	Leu	His	Asp	Ser	Ser	Ser	Ile	
			35					40					45				
10	TAT	TAC	CTA	GAT	AAA	CGT	CGC	CCG	GAT	TTA	GCA	AGC	TTA	ATG	CTC	AGC	192
	Tyr	Tyr	Leu	Asp	Lys	Arg	Arg	Pro	Asp	Leu	Ala	Ser	Leu	Met	Leu	Ser	
			50				55					60					
15	CAG	AAA	AAT	ATG	GAT	GAG	GAA	ATT	TCA	ACG	CTG	GCT	CTC	TCT	AAT	GAA	240
	Gln	Lys	Asn	Met	Asp	Glu	Glu	Ile	Ser	Thr	Leu	Ala	Leu	Ser	Asn	Glu	
						70					75					80	
20	TTG	TGC	CTT	GCC	GGG	ATC	GAA	ACA	AAA	ACA	GGA	AAA	TCA	CAA	GAT	GAA	288
	Leu	Cys	Leu	Ala	Gly	Ile	Glu	Thr	Lys	Thr	Gly	Lys	Ser	Gln	Asp	Glu	
					85					90					95		
25	GTG	ATG	GAT	ATG	TTG	TCA	ACT	TAT	CGT	TTA	AGT	GGA	GAG	ACA	CCT	TAT	336
	Val	Met	Asp	Met	Leu	Ser	Thr	Tyr	Arg	Leu	Ser	Gly	Glu	Thr	Pro	Tyr	
				100					105					110			
30	CAT	CAC	GCT	TAT	GAA	ACT	GTT	CGT	GAA	ATC	GTT	CAT	GAA	CGT	GAT	CCA	384
	His	His	Ala	Tyr	Glu	Thr	Val	Arg	Glu	Ile	Val	His	Glu	Arg	Asp	Pro	
			115					120					125				
35	GGA	TTT	CGT	CAT	TTG	TCA	CAG	GCA	CCC	ATT	GTT	GCT	GCT	AAG	CTC	GAT	432
	Gly	Phe	Arg	His	Leu	Ser	Gln	Ala	Pro	Ile	Val	Ala	Ala	Lys	Leu	Asp	
			130				135					140					
40	CCT	GTG	ACT	TTG	TTG	GGT	ATT	AGC	TCC	CAT	ATT	TCG	CCA	GAA	CTG	TAT	480
	Pro	Val	Thr	Leu	Leu	Gly	Ile	Ser	Ser	His	Ile	Ser	Pro	Glu	Leu	Tyr	
						150					155					160	
45	AAC	TTG	CTG	ATT	GAG	GAG	ATC	CCG	GAA	AAA	GAT	GAA	GCC	GCG	CTT	GAT	528
	Asn	Leu	Leu	Ile	Glu	Glu	Ile	Pro	Glu	Lys	Asp	Glu	Ala	Ala	Leu	Asp	
					165					170					175		
50	ACG	CTT	TAT	AAA	ACA	AAC	TTT	GGC	GAT	ATT	ACT	ACT	GCT	CAG	TTA	ATG	576
	Thr	Leu	Tyr	Lys	Thr	Asn	Phe	Gly	Asp	Ile	Thr	Thr	Ala	Gln	Leu	Met	
				180					185					190			
55	TCC	CCA	AGT	TAT	CTG	GCC	CGG	TAT	TAT	GGC	GTC	TCA	CCG	GAA	GAT	ATT	624
	Ser	Pro	Ser	Tyr	Leu	Ala	Arg	Tyr	Tyr	Gly	Val	Ser	Pro	Glu	Asp	Ile	
				195				200					205				
60	GCC	TAC	GTG	ACG	ACT	TCA	TTA	TCA	CAT	GTT	GGA	TAT	AGC	AGT	GAT	ATT	672
	Ala	Tyr	Val	Thr	Thr	Ser	Leu	Ser	His	Val	Gly	Tyr	Ser	Ser	Asp	Ile	
			210				215					220					
65	CTG	GTT	ATT	CCG	TTG	GTC	GAT	GGT	GTG	GGT	AAG	ATG	GAA	GTA	GTT	CGT	720
	Leu	Val	Ile	Pro	Leu	Val	Asp	Gly	Val	Gly	Lys	Met	Glu	Val	Val	Arg	
						230					235					240	
70	GTT	ACC	CGA	ACA	CCA	TCG	GAT	AAT	TAT	ACC	AGT	CAG	ACG	AAT	TAT	ATT	768
	Val	Thr	Arg	Thr	Pro	Ser	Asp	Asn	Tyr	Thr	Ser	Gln	Thr	Asn	Tyr	Ile	
					245					250					255		
75	GAG	CTG	TAT	CCA	CAG	GGT	GGC	GAC	AAT	TAT	TTG	ATC	AAA	TAC	AAT	CTA	816
	Glu	Leu	Tyr	Pro	Gln	Gly	Gly	Asp	Asn	Tyr	Leu	Ile	Lys	Tyr	Asn	Leu	
				260					265					270			
80	AGC	AAT	AGT	TTT	GGT	TTG	GAT	GAT	TTT	TAT	CTG	CAA	TAT	AAA	GAT	GGT	864
	Ser	Asn	Ser	Phe	Gly	Leu	Asp	Asp	Phe	Tyr	Leu	Gln	Tyr	Lys	Asp	Gly	
				275				280					285				
85	TCC	GCT	GAT	TGG	ACT	GAG	ATT	GCC	CAT	AAT	CCC	TAT	CCT	GAT	ATG	GTC	912
	Ser	Ala	Asp	Trp	Thr	Glu	Ile	Ala	His	Asn	Pro	Tyr	Pro	Asp	Met	Val	
			290				295					300					

	ATA	AAT	CAA	AAG	TAT	GAA	TCA	CAG	GCG	ACA	ATC	AAA	CGT	AGT	GAC	TCT	950
	Ile	Asn	Gln	Lys	Tyr	Glu	Ser	Gln	Ala	Thr	Ile	Lys	Arg	Ser	Asp	Ser	
	305					310					315					320	
5	GAC	AAT	ATA	CTC	AGT	ATA	GGG	TTA	CAA	AGA	TGG	CAT	AGC	GGT	AGT	TAT	1008
	Asp	Asn	Ile	Leu	Ser	Ile	Gly	Leu	Gln	Arg	Trp	His	Ser	Gly	Ser	Tyr	
					325					330					335		
10	AAT	TTT	GCC	GCC	GCC	AAT	TTT	AAA	ATT	GAC	CAA	TAC	TCC	CCG	AAA	GCT	1056
	Asn	Phe	Ala	Ala	Ala	Asn	Phe	Lys	Ile	Asp	Gln	Tyr	Ser	Pro	Lys	Ala	
				340					345					350			
15	TTC	CTG	CTT	AAA	ATG	AAT	AAG	GCT	ATT	CGG	TTG	CTC	AAA	GCT	ACC	GGC	1104
	Phe	Leu	Leu	Lys	Met	Asn	Lys	Ala	Ile	Arg	Leu	Leu	Lys	Ala	Thr	Gly	
			355					360					365				
20	CTC	TCT	TTT	GCT	ACG	TTG	GAG	CGT	ATT	GTT	GAT	AGT	GTT	AAT	AGC	ACC	1152
	Leu	Ser	Phe	Ala	Thr	Leu	Glu	Arg	Ile	Val	Asp	Ser	Val	Asn	Ser	Thr	
		370					375					380					
25	AAA	TCC	ATC	ACG	GTT	GAG	GTA	TTA	AAC	AAG	GTT	TAT	CGG	GTA	AAA	TTC	1200
	Lys	Ser	Ile	Thr	Val	Glu	Val	Leu	Asn	Lys	Val	Tyr	Arg	Val	Lys	Phe	
	385					390					395					400	
30	TAT	ATT	GAT	CGT	TAT	GGC	ATC	AGT	GAA	GAG	ACA	GCC	GCT	ATT	TTG	GCT	1248
	Tyr	Ile	Asp	Arg	Tyr	Gly	Ile	Ser	Glu	Thr	Ala	Ala	Ile	Leu	Ala		
					405					410				415			
35	AAT	ATT	AAT	ATC	TCT	CAG	CAA	GCT	GTT	GGC	AAT	CAG	CTT	AGC	CAG	TTT	1296
	Asn	Ile	Asn	Ile	Ser	Gln	Gln	Ala	Val	Gly	Asn	Gln	Leu	Ser	Gln	Phe	
				420					425					430			
40	GAG	CAA	CTA	TTT	AAT	CAC	CCG	CCG	CTC	AAT	GGT	ATT	CGC	TAT	GAA	ATC	1344
	Glu	Gln	Leu	Phe	Asn	His	Pro	Pro	Leu	Asn	Gly	Ile	Arg	Tyr	Glu	Ile	
			435					440					445				
45	AGT	GAG	GAC	AAC	TCC	AAA	CAT	CTT	CCT	AAT	CCT	GAT	CTG	AAC	CTT	AAA	1392
	Ser	Glu	Asp	Asn	Ser	Lys	His	Leu	Pro	Asn	Pro	Asp	Leu	Asn	Leu	Lys	
		450					455					460					
50	CCA	GAC	AGT	ACC	GGT	GAT	GAT	CAA	CGC	AAG	GCG	GTT	TTA	AAA	CGC	GCG	1440
	Pro	Asp	Ser	Thr	Gly	Asp	Asp	Gln	Arg	Lys	Ala	Val	Leu	Lys	Arg	Ala	
	465				470						475					480	
55	TTT	CAG	GTT	AAC	GCC	AGT	GAG	TTG	TAT	CAG	ATG	TTA	TTG	ATC	ACT	GAT	1488
	Phe	Gln	Val	Asn	Ala	Ser	Glu	Leu	Tyr	Gln	Met	Leu	Leu	Ile	Thr	Asp	
					485				490					495			
60	CGT	AAA	GAA	GAC	GGT	GTT	ATC	AAA	AAT	AAC	TTA	GAG	AAT	TTG	TCT	GAT	1536
	Arg	Lys	Glu	Gly	Gly	Val	Ile	Lys	Asn	Asn	Leu	Glu	Asn	Leu	Ser	Asp	
				500				505						510			
65	CTG	TAT	TTG	GTT	AGT	TTG	CTG	GCC	CAG	ATT	CAT	AAC	CTG	ACT	ATT	GCT	1584
	Leu	Tyr	Leu	Val	Ser	Leu	Leu	Ala	Gln	Ile	His	Asn	Leu	Thr	Ile	Ala	
			515					520					525				
70	GAA	TTG	AAC	ATT	TTG	TTG	GTG	ATT	TGT	GCG	TAT	GGC	GAC	ACC	AAC	ATT	1632
	Glu	Leu	Asn	Ile	Leu	Leu	Val	Ile	Cys	Gly	Tyr	Gly	Asp	Thr	Asn	Ile	
		530					535					540					
75	TAT	CAG	ATT	ACC	GAC	GAT	AAT	TTA	GCC	AAA	ATA	GTG	GAA	ACA	TTG	TTG	1680
	Tyr	Gln	Ile	Thr	Asp	Asp	Asn	Leu	Ala	Lys	Ile	Val	Glu	Thr	Leu	Leu	
	545					550					555					560	
80	TGG	ATC	ACT	CAA	TGG	TTG	AAG	ACC	CAA	AAA	TGG	ACA	GTT	ACC	GAC	CTG	1728
	Trp	Ile	Thr	Gln	Trp	Leu	Lys	Thr	Gln	Lys	Trp	Thr	Val	Thr	Asp	Leu	
					565					570					575		
85	TTT	CTG	ATG	ACC	ACG	GCC	ACT	TAC	AGC	ACC	ACT	TTA	ACG	CCA	GAA	ATT	1776
	Phe	Leu	Met	Thr	Thr	Ala	Thr	Tyr	Ser	Thr	Thr	Leu	Thr	Pro	Glu	Ile	
				580					585					590			

	AGC	AAT	CTG	ACG	GCT	ACG	TTG	TCT	TCA	ACT	TTG	CAT	GGC	AAA	GAG	AGT	1824
	Ser	Asn	Leu	Thr	Ala	Thr	Leu	Ser	Ser	Thr	Leu	His	Gly	Lys	Glu	Ser	
			595					600					605				
5	CTG	ATT	GGG	GAA	GAT	CTG	AAA	AGA	GCA	ATG	GCG	CCT	TGC	TTC	ACT	TCG	1872
	Leu	Ile	Gly	Glu	Asp	Leu	Lys	Arg	Ala	Met	Ala	Pro	Cys	Phe	Thr	Ser	
		610					615					620					
10	GCT	TTG	CAT	TTG	ACT	TCT	CAA	GAA	GTT	GCG	TAT	GAC	CTG	CTG	TTG	TGG	1920
	Ala	Leu	His	Leu	Thr	Ser	Gln	Glu	Val	Ala	Tyr	Asp	Leu	Leu	Leu	Trp	
		625				630					635					640	
15	ATA	GAC	CAG	ATT	CAA	CCG	GCA	CAA	ATA	ACT	GTT	GAT	GGG	TTT	TGG	GAA	1968
	Ile	Asp	Gln	Ile	Gln	Pro	Ala	Gln	Ile	Thr	Val	Asp	Gly	Phe	Trp	Glu	
					645					650					655		
20	GAA	GTG	CAA	ACA	ACA	CCA	ACC	AGC	TTG	AAG	GTG	ATT	ACC	TTT	GCT	CAG	2016
	Glu	Val	Gln	Thr	Thr	Pro	Thr	Ser	Leu	Lys	Val	Ile	Thr	Phe	Ala	Gln	
					660					665				670			
25	GTG	CTG	GCA	CAA	TTG	AGC	CTG	ATC	TAT	CGT	CGT	ATT	GGG	TTA	AGT	GAA	2064
	Val	Leu	Ala	Gln	Leu	Ser	Leu	Ile	Tyr	Arg	Arg	Ile	Gly	Leu	Ser	Glu	
			675					680					685				
30	ACG	GAA	CTG	TCA	CTG	ATC	GTG	ACT	CAA	TCT	TCT	CTG	CTA	GTG	GCA	GGC	2112
	Thr	Glu	Leu	Ser	Leu	Ile	Val	Thr	Gln	Ser	Ser	Leu	Leu	Val	Ala	Gly	
		690					695					700					
35	AAA	AGC	ATA	CTG	GAT	CAC	GGT	CTG	TTA	ACC	CTG	ATG	GCC	TTG	GAA	GGT	2160
	Lys	Ser	Ile	Leu	Asp	His	Gly	Leu	Leu	Thr	Leu	Met	Ala	Leu	Glu	Gly	
		705				710					715				720		
40	TTT	CAT	ACC	TGG	GTT	AAT	GGC	TTG	GGG	CAA	CAT	GCC	TCC	TTG	ATA	TTG	2208
	Phe	His	Thr	Trp	Val	Asn	Gly	Leu	Gly	Gln	His	Ala	Ser	Leu	Ile	Leu	
					725					730					735		
45	GCG	GCG	TTG	AAA	GAC	GGA	GCC	TTG	ACA	GTT	ACC	GAT	GTA	GCA	CAA	GCT	2256
	Ala	Ala	Leu	Lys	Asp	Gly	Ala	Leu	Thr	Val	Thr	Asp	Val	Ala	Gln	Ala	
				740					745				750				
50	ATG	AAT	AAG	GAG	GAA	TCT	CTC	CTA	CAA	ATG	GCA	GCT	AAT	CAG	GTG	GAG	2304
	Met	Asn	Lys	Glu	Glu	Ser	Leu	Leu	Gln	Met	Ala	Ala	Asn	Gln	Val	Glu	
			755					760					765				
55	AAG	GAT	CTA	ACA	AAA	CTG	ACC	AGT	TGG	ACA	CAG	ATT	GAC	GCT	ATT	CTG	2352
	Lys	Asp	Leu	Thr	Lys	Leu	Thr	Ser	Trp	Thr	Gln	Ile	Asp	Ala	Ile	Leu	
		770					775					780					
60	CAA	TGG	TTA	CAG	ATG	TCT	TCG	GCC	TTG	GCG	GTT	TCT	CCA	CTG	GAT	CTG	2400
	Gln	Trp	Leu	Gln	Met	Ser	Ser	Ala	Leu	Ala	Val	Ser	Pro	Leu	Asp	Leu	
		785				790					795				800		
65	GCA	GGG	ATG	ATG	GCC	CTG	AAA	TAT	GGG	ATA	GAT	CAT	AAC	TAT	GCT	GCC	2448
	Ala	Gly	Met	Met	Ala	Leu	Lys	Tyr	Gly	Ile	Asp	His	Asn	Tyr	Ala	Ala	
					805					810					815		
70	TGG	CAA	GCT	GCG	GCG	GCT	GCG	CTG	ATG	GCT	GAT	CAT	GCT	AAT	CAG	GCA	2496
	Trp	Gln	Ala	Ala	Ala	Ala	Ala	Leu	Met	Ala	Asp	His	Ala	Asn	Gln	Ala	
				820					825					830			
75	CAG	AAA	AAA	CTG	GAT	GAG	ACG	TTC	AGT	AAG	GCA	TTA	TGT	AAC	TAT	TAT	2544
	Gln	Lys	Lys	Leu	Asp	Glu	Thr	Phe	Ser	Lys	Ala	Leu	Cys	Asn	Tyr	Tyr	
			835					840					845				
80	ATT	AAT	GCT	GTT	GTC	GAT	AGT	GCT	GCT	GGA	GTA	CGT	GAT	CGT	AAC	GGT	2592
	Ile	Asn	Ala	Val	Val	Asp	Ser	Ala	Ala	Gly	Val	Arg	Asp	Arg	Asn	Gly	
		850					855					860					
85	TTA	TAT	ACC	TAT	TTG	CTG	ATT	GAT	AAT	CAG	GTT	TCT	GCC	GAT	GTG	ATC	2640
	Leu	Tyr	Thr	Tyr	Leu	Leu	Ile	Asp	Asn	Gln	Val	Ser	Ala	Asp	Val	Ile	

	365				370					375					380		
5	ACT	TCA	CGT	ATT	GCA	GAA	GCT	ATC	CCC	GGT	ATT	CAA	CTG	TAC	GTT	AAC	2688
	Thr	Ser	Arg	Ile	Ala	Glu	Ala	Ile	Ala	Gly	Ile	Gln	Leu	Tyr	Val	Asn	
					885					890					895		
10	CGG	GCT	TTA	AAC	CGA	GAT	GAA	GCT	CAG	CTT	GCA	TCG	GAC	GTT	AGT	ACC	2736
	Arg	Ala	Leu	Asn	Arg	Asp	Glu	Gly	Gln	Leu	Ala	Ser	Asp	Val	Ser	Thr	
				900					905					910			
15	CGT	CAG	TTC	TTC	ACT	GAC	TGG	GAA	CGT	TAC	AAT	AAA	CGT	TAC	AGT	ACT	2784
	Arg	Gln	Phe	Phe	Thr	Asp	Trp	Glu	Arg	Tyr	Asn	Lys	Arg	Tyr	Ser	Thr	
			915				920						925				
20	TGG	GCT	GGT	GTC	TCT	GAA	CTG	GTC	TAT	TAT	CCA	GAA	AAC	TAT	GTT	GAT	2832
	Trp	Ala	Gly	Val	Ser	Glu	Leu	Val	Tyr	Tyr	Pro	Glu	Asn	Tyr	Val	Asp	
		930					935					940					
25	CCC	ACT	CAG	CGC	ATT	GGG	CAA	ACC	AAA	ATG	ATG	GAT	GCG	CTG	TTG	CAA	2880
	Pro	Thr	Gln	Arg	Ile	Gly	Gln	Thr	Lys	Met	Met	Asp	Ala	Leu	Leu	Gln	
		945				950				955						960	
30	TCC	ATC	AAC	CAG	AGC	CAG	CTA	AAT	GCG	GAT	ACG	GTG	GAA	GAT	GCT	TTC	2928
	Ser	Ile	Asn	Gln	Ser	Gln	Leu	Asn	Ala	Asp	Thr	Val	Glu	Asp	Ala	Phe	
					965				970						975		
35	AAA	ACT	TAT	TTG	ACC	AGC	TTT	GAG	CAG	GTA	GCA	AAT	CTG	AAA	GTA	ATT	2976
	Lys	Thr	Tyr	Leu	Thr	Ser	Phe	Glu	Gln	Val	Ala	Asn	Leu	Lys	Val	Ile	
				980				985					990				
40	AGT	GCT	TAC	CAC	GAT	AAT	GTG	AAT	GTG	GAT	CAA	GGA	TTA	ACT	TAT	TTT	3024
	Ser	Ala	Tyr	His	Asp	Asn	Val	Asn	Val	Asp	Gln	Gly	Leu	Thr	Tyr	Phe	
			995				1000					1005					
45	ATC	GGT	ATC	GAC	CAA	GCA	GCT	CCG	GGT	ACG	TAT	TAC	TGG	CGT	AGT	GTT	3072
	Ile	Gly	Ile	Asp	Gln	Ala	Ala	Pro	Gly	Thr	Tyr	Tyr	Trp	Arg	Ser	Val	
		1010					1015					1020					
50	GAT	CAC	AGC	AAA	TGT	GAA	AAT	GCC	AAG	TTT	GCC	GCT	AAT	GCT	TGG	GGT	3120
	Asp	His	Ser	Lys	Cys	Glu	Asn	Gly	Lys	Phe	Ala	Ala	Asn	Ala	Trp	Gly	
		1025				1030				1035						1040	
55	GAG	TGG	AAT	AAA	ATT	ACC	TGT	GCT	GTC	AAT	CCT	TGG	AAA	AAT	ATC	ATC	3168
	Glu	Trp	Asn	Lys	Ile	Thr	Cys	Ala	Val	Asn	Pro	Trp	Lys	Asn	Ile	Ile	
				1045					1050						1055		
60	CGT	CCG	GTT	GTT	TAT	ATG	TCC	CGC	TTA	TAT	CTG	CTA	TGG	CTG	GAG	CAG	3216
	Arg	Pro	Val	Val	Tyr	Met	Ser	Arg	Leu	Tyr	Leu	Leu	Trp	Leu	Glu	Gln	
				1060					1065					1070			
65	CAA	TCA	AAG	AAA	AGT	GAT	GAT	GGT	AAA	ACC	ACG	ATT	TAT	CAA	TAT	AAC	3264
	Gln	Ser	Lys	Lys	Ser	Asp	Asp	Gly	Lys	Thr	Thr	Ile	Tyr	Gln	Tyr	Asn	
			1075					1080					1085				
70	TTA	AAA	CTG	GCT	CAT	ATT	CGT	TAC	GAC	GGT	AGT	TGG	AAT	ACA	CCA	TTT	3312
	Leu	Lys	Leu	Ala	His	Ile	Arg	Tyr	Asp	Gly	Ser	Trp	Asn	Thr	Pro	Phe	
		1090					1095					1100					
75	ACT	TTT	GAT	GTG	ACA	GAA	AAG	GTA	AAA	AAT	TAC	ACG	TCG	AGT	ACT	GAT	3360
	Thr	Phe	Asp	Val	Thr	Glu	Lys	Val	Lys	Asn	Tyr	Thr	Ser	Ser	Thr	Asp	
		1105				1110					1115					1120	
80	GCT	GCT	GAA	TCT	TTA	GGG	TTG	TAT	TGT	ACT	GGT	TAT	CAA	GGG	GAA	GAC	3408
	Ala	Ala	Glu	Ser	Leu	Gly	Leu	Tyr	Cys	Thr	Gly	Tyr	Gln	Gly	Glu	Asp	
					1125				1130					1135			
85	ACT	CTA	TTA	GTT	ATG	TTC	TAT	TCG	ATG	CAG	AGT	AGT	TAT	AGC	TCC	TAT	3456
	Thr	Leu	Leu	Val	Met	Phe	Tyr	Ser	Met	Gln	Ser	Ser	Tyr	Ser	Ser	Tyr	
				1140					1145					1150			
90	ACC	GAT	AAT	AAT	GCG	CCG	GTC	ACT	GGG	CTA	TAT	ATT	TTC	GCT	GAT	ATG	3504

	Thr	Asp	Asn	Asn	Ala	Pro	Val	Thr	Gly	Leu	Tyr	Ile	Phe	Ala	Asp	Met	
			1155					1160					1165				
5	TCA	TCA	GAC	AAT	ATG	ACG	AAT	GCA	CAA	GCA	ACT	AAC	TAT	TGG	AAT	AAC	3550
	Ser	Ser	Asp	Asn	Met	Thr	Asn	Ala	Gln	Ala	Thr	Asn	Tyr	Trp	Asn	Asn	
			1170				1175					1180					
10	AGT	TAT	CCG	CAA	TTT	GAT	ACT	GTG	ATG	GCA	GAT	CCG	GAT	AGC	GAC	AAT	3600
	Ser	Tyr	Pro	Gln	Phe	Asp	Thr	Val	Met	Ala	Asp	Pro	Asp	Ser	Asp	Asn	
			1185				1190					1195				1200	
15	AAA	AAA	GTC	ATA	ACC	AGA	AGA	GTT	AAT	AAC	CGT	TAT	GCG	GAG	GAT	TAT	3648
	Lys	Lys	Val	Ile	Thr	Arg	Arg	Val	Asn	Asn	Arg	Tyr	Ala	Glu	Asp	Tyr	
					1205					1210					1215		
20	GAA	ATT	CCT	TCC	TCT	GTG	ACA	AGT	AAC	AGT	AAT	TAT	TCT	TGG	GGT	GAT	3696
	Glu	Ile	Pro	Ser	Ser	Val	Thr	Ser	Asn	Ser	Asn	Tyr	Ser	Trp	Gly	Asp	
				1220					1225					1230			
25	CAC	AGT	TTA	ACC	ATG	CTT	TAT	GGT	GGT	AGT	GTT	CCT	AAT	ATT	ACT	TTT	3744
	His	Ser	Leu	Thr	Met	Leu	Tyr	Gly	Gly	Ser	Val	Pro	Asn	Ile	Thr	Phe	
				1235				1240					1245				
30	GAA	TCG	GCG	GCA	GAA	GAT	TTA	AGG	CTA	TCT	ACC	AAT	ATG	GCA	TTG	AGT	3792
	Glu	Ser	Ala	Ala	Glu	Asp	Leu	Arg	Leu	Ser	Thr	Asn	Met	Ala	Leu	Ser	
			1250				1255					1260					
35	ATT	ATT	CAT	AAT	GGA	TAT	GCG	GGA	ACC	CGC	CGT	ATA	CAA	TGT	AAT	CTT	3840
	Ile	Ile	His	Asn	Gly	Tyr	Ala	Gly	Thr	Arg	Arg	Ile	Gln	Cys	Asn	Leu	
			1265			1270					1275					1280	
40	ATG	AAA	CAA	TAC	GCT	TCA	TTA	GGT	GAT	AAA	TTT	ATA	ATT	TAT	GAT	TCA	3888
	Met	Lys	Gln	Tyr	Ala	Ser	Leu	Gly	Asp	Lys	Phe	Ile	Ile	Tyr	Asp	Ser	
				1285					1290						1295		
45	TCA	TTT	GAT	GAT	GCA	AAC	CGT	TTT	AAT	CTG	GTG	CCA	TTG	TTT	AAA	TTC	3936
	Ser	Phe	Asp	Asp	Ala	Asn	Arg	Phe	Asn	Leu	Val	Pro	Leu	Phe	Lys	Phe	
				1300				1305					1310				
50	GGA	AAA	GAC	GAG	AAC	TCA	GAT	GAT	AGT	ATT	TGT	ATA	TAT	AAT	GAA	AAC	3984
	Gly	Lys	Asp	Glu	Asn	Ser	Asp	Asp	Ser	Ile	Cys	Ile	Tyr	Asn	Glu	Asn	
			1315				1320					1325					
55	CCT	TCC	TCT	GAA	GAT	AAG	AAG	TGG	TAT	TTT	TCT	TCG	AAA	GAT	GAC	AAT	4032
	Pro	Ser	Ser	Glu	Asp	Lys	Lys	Trp	Tyr	Phe	Ser	Ser	Lys	Asp	Asp	Asn	
			1330				1335					1340					
60	AAA	ACA	GCG	GAT	TAT	AAT	GGT	GGA	ACT	CAA	TGT	ATA	GAT	GCT	GGA	ACC	4080
	Lys	Thr	Ala	Asp	Tyr	Asn	Gly	Gly	Thr	Gln	Cys	Ile	Asp	Ala	Gly	Thr	
			1345			1350				1355					1360		
65	AGT	AAC	AAA	GAT	TTT	TAT	TAT	AAT	CTC	CAG	GAG	ATT	GAA	GTA	ATT	AGT	4128
	Ser	Asn	Lys	Asp	Phe	Tyr	Tyr	Asn	Leu	Gln	Glu	Ile	Glu	Val	Ile	Ser	
				1365				1370					1375				
70	GTT	ACT	GGT	GGG	TAT	TGG	TCG	AGT	TAT	AAA	ATA	TCC	AAC	CCG	ATT	AAT	4176
	Val	Thr	Gly	Gly	Tyr	Trp	Ser	Ser	Tyr	Lys	Ile	Ser	Asn	Pro	Ile	Asn	
			1380					1385					1390				
75	ATC	AAT	ACG	GGC	ATT	GAT	AGT	GCT	AAA	GTA	AAA	GTC	ACC	GTA	AAA	GCG	4224
	Ile	Asn	Thr	Gly	Ile	Asp	Ser	Ala	Lys	Val	Lys	Val	Thr	Val	Lys	Ala	
			1395				1400					1405					
80	GGT	GGT	GAC	GAT	CAA	ATC	TTT	ACT	GCT	GAT	AAT	AGT	ACC	TAT	GTT	CCT	4272
	Gly	Gly	Asp	Asp	Gln	Ile	Phe	Thr	Ala	Asp	Asn	Ser	Thr	Tyr	Val	Pro	
			1410			1415					1420						
85	CAG	CAA	CCG	GCA	CCC	AGT	TTT	GAG	GAG	ATG	ATT	TAT	CAG	TTC	AAT	AAC	4320
	Gln	Gln	Pro	Ala	Pro	Ser	Phe	Glu	Glu	Met	Ile	Tyr	Gln	Phe	Asn	Asn	
			1425			1430				1435						1440	

	CTG	ACA	ATA	GAT	TGT	AAG	AAT	TTA	AAT	TTC	ATC	GAC	AAT	CAG	GCA	GAT	4163
	Leu	Thr	Ile	Asp	Cys	Lys	Asn	Leu	Asn	Phe	Ile	Asp	Asn	Gln	Ala	His	
					1445					1450					1455		
5	ATT	GAG	ATT	GAT	TTC	ACC	GCT	ACG	GCA	CAA	GAT	GGC	CGA	TTC	TTG	GGT	4416
	Ile	Glu	Ile	Asp	Phe	Thr	Ala	Thr	Ala	Gln	Asp	Gly	Arg	Phe	Leu	Gly	
					1460					1465					1470		
10	GCA	GAA	ACT	TTT	ATT	ATC	CCG	GTA	ACT	AAA	AAA	GTT	CTC	GGT	ACT	GAG	4464
	Ala	Glu	Thr	Phe	Ile	Ile	Pro	Val	Thr	Lys	Lys	Val	Leu	Gly	Thr	Glu	
					1475					1480					1485		
15	AAC	GTG	ATT	GCG	TTA	TAT	AGC	GAA	AAT	AAC	GGT	GTT	CAA	TAT	ATG	CAA	4512
	Asn	Val	Ile	Ala	Leu	Tyr	Ser	Glu	Asn	Asn	Gly	Val	Gln	Tyr	Met	Gln	
					1490					1495					1500		
20	ATT	GGC	GCA	TAT	CGT	ACC	CGT	TTG	AAT	ACG	TTA	TTC	GCT	CAA	CAG	TTG	4560
	Ile	Gly	Ala	Tyr	Arg	Thr	Arg	Leu	Asn	Thr	Leu	Phe	Ala	Gln	Gln	Leu	
					1505					1510					1515		1520
25	GTT	AGC	CGT	GCT	AAT	CGT	GGC	ATT	GAT	GCA	GTG	CTC	AGT	ATG	GAA	ACT	4608
	Val	Ser	Arg	Ala	Asn	Arg	Gly	Ile	Asp	Ala	Val	Leu	Ser	Met	Glu	Thr	
					1525					1530					1535		
30	CAG	AAT	ATT	CAG	GAA	CCG	CAA	TTA	GGA	GCG	GGC	ACA	TAT	GTG	CAG	CTT	4656
	Gln	Asn	Ile	Gln	Glu	Pro	Gln	Leu	Gly	Ala	Gly	Thr	Tyr	Val	Gln	Leu	
					1540					1545					1550		
35	GTG	TTG	GAT	AAA	TAT	GAT	GAG	TCT	ATT	CAT	GGC	ACT	AAT	AAA	AGC	TTT	4704
	Val	Leu	Asp	Lys	Tyr	Asp	Glu	Ser	Ile	His	Gly	Thr	Asn	Lys	Ser	Phe	
					1555					1560					1565		
40	GCT	ATT	GAA	TAT	GTT	GAT	ATA	TTT	AAA	GAG	AAC	GAT	AGT	TTT	GTG	ATT	4752
	Ala	Ile	Glu	Tyr	Val	Asp	Ile	Phe	Lys	Glu	Asn	Asp	Ser	Phe	Val	Ile	
					1570					1575					1580		
45	TAT	CAA	GGA	GAA	CTT	AGC	GAA	ACA	AGT	CAA	ACT	GTT	GTG	AAA	GTT	TTC	4800
	Tyr	Gln	Gly	Glu	Leu	Ser	Glu	Thr	Ser	Gln	Thr	Val	Val	Lys	Val	Phe	
					1585					1590					1595		1600
50	TTA	TCC	TAT	TTT	ATA	GAG	GCG	ACT	GGA	AAT	AAG	AAC	CAC	TTA	TGG	GTA	4848
	Leu	Ser	Tyr	Phe	Ile	Glu	Ala	Thr	Gly	Asn	Lys	Asn	His	Leu	Trp	Val	
					1605					1610					1615		
55	CGT	GCT	AAA	TAC	CAA	AAG	GAA	ACG	ACT	GAT	AAG	ATC	TTG	TTC	GAC	CGT	4896
	Arg	Ala	Lys	Tyr	Gln	Lys	Glu	Thr	Thr	Asp	Lys	Ile	Leu	Phe	Asp	Arg	
					1620					1625					1630		
60	ACT	GAT	GAG	AAA	GAT	CCG	CAC	GGT	TGG	TTT	CTC	AGC	GAC	GAT	CAC	AAG	4944
	Thr	Asp	Glu	Lys	Asp	Pro	His	Gly	Trp	Phe	Leu	Ser	Asp	Asp	His	Lys	
					1635					1640					1645		
65	ACC	TTT	AGT	GGT	CTC	TCT	TCC	GCA	CAG	GCA	TTA	AAG	AAC	GAC	AGT	GAA	4992
	Thr	Phe	Ser	Gly	Leu	Ser	Ser	Ala	Gln	Ala	Leu	Lys	Asn	Asp	Ser	Glu	
					1650					1655					1660		
70	CCG	ATG	GAT	TTC	TCT	GGC	GCC	AAT	GCT	CTC	TAT	TTC	TGG	GAA	CTG	TTC	5040
	Pro	Met	Asp	Phe	Ser	Gly	Ala	Asn	Ala	Leu	Tyr	Phe	Trp	Glu	Leu	Phe	
					1665					1670					1675		1680
75	TAT	TAC	ACG	CCG	ATG	ATG	ATG	GCT	CAT	CGT	TTG	TTG	CAG	GAA	CAG	AAT	5088
	Tyr	Tyr	Thr	Pro	Met	Met	Met	Ala	His	Arg	Leu	Leu	Gln	Glu	Gln	Asn	
					1685					1690					1695		
80	TTT	GAT	GCG	GCG	AAC	CAT	TGG	TTC	CGT	TAT	GTC	TGG	AGT	CCA	TCC	GGT	5136
	Phe	Asp	Ala	Ala	Asn	His	Trp	Phe	Arg	Tyr	Val	Trp	Ser	Pro	Ser	Gly	
					1700					1705					1710		
85	TAT	ATC	GTT	GAT	GGT	AAA	ATT	GCT	ATC	TAC	CAC	TGG	AAC	GTG	CGA	CCG	5184
	Tyr	Ile	Val	Asp	Gly	Lys	Ile	Ala	Ile	Tyr	His	Trp	Asn	Val	Arg	Pro	
					1715					1720					1725		

CTG GAA GAA GAC ACC AGT TGG AAT GCA CAA CAA CTG GAC TCC ACC GAT 5211  
 Leu Glu Glu Asp Thr Ser Trp Asn Ala Gln Gln Leu Asp Ser Thr Asp  
 1730 1735 1740  
 5 CCA GAT GCT GTA GCC CAA GAT GAT CCG ATG CAC TAC AAG GTG GCT ACC 5280  
 Pro Asp Ala Val Ala Gln Asp Asp Pro Met His Tyr Lys Val Ala Thr  
 1745 1750 1755 1760  
 10 TTT ATG GCG ACG TTG GAT CTG CTA ATG GCC CGT GGT GAT GCT GCT TAC 5328  
 Phe Met Ala Thr Leu Asp Leu Leu Met Ala Arg Gly Asp Ala Ala Tyr  
 1765 1770 1775  
 15 CGC CAG TTA GAG CGT GAT ACG TTG GCT GAA GCT AAA ATG TGG TAT ACA 5376  
 Arg Gln Leu Glu Arg Asp Thr Leu Ala Glu Ala Lys Met Trp Tyr Thr  
 1780 1785 1790  
 20 CAG GCG CTT AAT CTG TTG GGT GAT GAG CCA CAA GTG ATG CTG AGT ACG 5424  
 Gln Ala Leu Asn Leu Leu Gly Asp Glu Pro Gln Val Met Leu Ser Thr  
 1795 1800 1805  
 ACT TGG GCT AAT CCA ACA TTG GGT AAT GCT GCT TCA AAA ACC ACA CAG 5472  
 Thr Trp Ala Asn Pro Thr Leu Gly Asn Ala Ala Ser Lys Thr Thr Gln  
 1810 1815 1820  
 25 CAG GTT CGT CAG CAA GTG CTT ACC CAG TTG CGT CTC AAT AGC AGG GTA 5520  
 Gln Val Arg Gln Gln Val Leu Thr Gln Leu Arg Leu Asn Ser Arg Val  
 1825 1830 1835 1840  
 30 AAA ACC CCG TTG 5532  
 Lys Thr Pro Leu  
 1844

35 (2) INFORMATION FOR SEQ ID NO:53:  
 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1844 amino acids  
 (B) TYPE: amino acids  
 (C) STRANDEDNESS: single  
 40 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:53 (TcbA<sub>ii</sub>):

Features	From	To	Description
Peptide	1	1844	TcbA <sub>ii</sub> peptide
Fragment	1	11	(SEQ ID NO:1)
Fragment	978	990	(SEQ ID NO:23)
Fragment	1387	1401	(SEQ ID NO:22)
Fragment	1484	1505	(SEQ ID NO:24)
Fragment	1527	1552	(SEQ ID NO:21)

55 Phe Ile Gln Gly Tyr Ser Asp Leu Phe Gly Asn Arg Ala Asp Asn Tyr  
 1 5 10 15  
 Ala Ala Pro Gly Ser Val Ala Ser Met Phe Ser Pro Ala Ala Tyr Leu  
 20 25 30  
 60 Thr Glu Leu Tyr Arg Glu Ala Lys Asn Leu His Asp Ser Ser Ser Ile  
 35 40 45  
 Tyr Tyr Leu Asp Lys Arg Arg Pro Asp Leu Ala Ser Leu Met Leu Ser  
 50 55 60  
 65 Gln Lys Asn Met Asp Glu Glu Ile Ser Thr Leu Ala Leu Ser Asn Glu  
 65 70 75 80  
 Leu Cys Leu Ala Gly Ile Glu Thr Lys Thr Gly Lys Ser Gln Asp Glu

				35					90					35				
		Val	Met	Asp	Met	Leu	Ser	Thr	Tyr	Arg	Leu	Ser	Gly	Glu	Thr	Pro	Tyr	
				100						105					110			
5		His	His	Ala	Tyr	Glu	Thr	Val	Arg	Glu	Ile	Val	His	Glu	Arg	Asp	Pro	
				115					120					125				
		Gly	Phe	Arg	His	Leu	Ser	Gln	Ala	Pro	Ile	Val	Ala	Ala	Lys	Leu	Asp	
10			130					135					140					
		Pro	Val	Thr	Leu	Leu	Gly	Ile	Ser	Ser	His	Ile	Ser	Pro	Glu	Leu	Tyr	
			145				150					155					160	
15		Asn	Leu	Leu	Ile	Glu	Glu	Ile	Pro	Glu	Lys	Asp	Glu	Ala	Ala	Leu	Asp	
					165						170						175	
		Thr	Leu	Tyr	Lys	Thr	Asn	Phe	Gly	Asp	Ile	Thr	Thr	Ala	Gln	Leu	Met	
					180					185					190			
20		Ser	Pro	Ser	Tyr	Leu	Ala	Arg	Tyr	Tyr	Gly	Val	Ser	Pro	Glu	Asp	Ile	
				195					200					205				
		Ala	Tyr	Val	Thr	Thr	Ser	Leu	Ser	His	Val	Gly	Tyr	Ser	Ser	Asp	Ile	
25			210					215					220					
		Leu	Val	Ile	Pro	Leu	Val	Asp	Gly	Val	Gly	Lys	Met	Glu	Val	Val	Arg	
			225				230					235					240	
30		Val	Thr	Arg	Thr	Pro	Ser	Asp	Asn	Tyr	Thr	Ser	Gln	Thr	Asn	Tyr	Ile	
					245						250					255		
		Glu	Leu	Tyr	Pro	Gln	Gly	Gly	Asp	Asn	Tyr	Leu	Ile	Lys	Tyr	Asn	Leu	
					260				265						270			
35		Ser	Asn	Ser	Phe	Gly	Leu	Asp	Asp	Phe	Tyr	Leu	Gln	Tyr	Lys	Asp	Gly	
				275					280					285				
		Ser	Ala	Asp	Trp	Thr	Glu	Ile	Ala	His	Asn	Pro	Tyr	Pro	Asp	Met	Val	
40			290					295					300					
		Ile	Asn	Gln	Lys	Tyr	Glu	Ser	Gln	Ala	Thr	Ile	Lys	Arg	Ser	Asp	Ser	
			305				310					315					320	
45		Asp	Asn	Ile	Leu	Ser	Ile	Gly	Leu	Gln	Arg	Trp	His	Ser	Gly	Ser	Tyr	
					325						330					335		
		Asn	Phe	Ala	Ala	Ala	Asn	Phe	Lys	Ile	Asp	Gln	Tyr	Ser	Pro	Lys	Ala	
					340					345					350			
50		Phe	Leu	Leu	Lys	Met	Asn	Lys	Ala	Ile	Arg	Leu	Leu	Lys	Ala	Thr	Gly	
				355					360					365				
		Leu	Ser	Phe	Ala	Thr	Leu	Glu	Arg	Ile	Val	Asp	Ser	Val	Asn	Ser	Thr	
55			370					375					380					
		Lys	Ser	Ile	Thr	Val	Glu	Val	Leu	Asn	Lys	Val	Tyr	Arg	Val	Lys	Phe	
			385				390					395					400	
60		Tyr	Ile	Asp	Arg	Tyr	Gly	Ile	Ser	Glu	Glu	Thr	Ala	Ala	Ile	Leu	Ala	
					405						410					415		
		Asn	Ile	Asn	Ile	Ser	Gln	Gln	Ala	Val	Gly	Asn	Gln	Leu	Ser	Gln	Phe	
					420					425					430			
65		Glu	Gln	Leu	Phe	Asn	His	Pro	Pro	Leu	Asn	Gly	Ile	Arg	Tyr	Glu	Ile	
				435					440					445				
		Ser	Glu	Asp	Asn	Ser	Lys	His	Leu	Pro	Asn	Pro	Asp	Leu	Asn	Leu	Lys	
70			450					455					460					



Pro Asp Ser Thr Gly Asp Asp Gln Arg Lys Ala Val Leu Lys Arg Ala  
 465 470 475 480  
 5 Phe Gln Val Asn Ala Ser Glu Leu Tyr Gln Met Leu Leu Ile Thr Asp  
 485 490 495  
 Arg Lys Glu Asp Gly Val Ile Lys Asn Asn Leu Glu Asn Leu Ser Asp  
 500 505 510  
 10 Leu Tyr Leu Val Ser Leu Leu Ala Gln Ile His Asn Leu Thr Ile Ala  
 515 520 525  
 Glu Leu Asn Ile Leu Leu Val Ile Cys Gly Tyr Gly Asp Thr Asn Ile  
 530 535 540  
 15 Tyr Gln Ile Thr Asp Asp Asn Leu Ala Lys Ile Val Glu Thr Leu Leu  
 545 550 555 560  
 20 Trp Ile Thr Gln Trp Leu Lys Thr Gln Lys Trp Thr Val Thr Asp Leu  
 565 570 575  
 Phe Leu Met Thr Thr Ala Thr Tyr Ser Thr Thr Leu Thr Pro Glu Ile  
 580 585 590  
 25 Ser Asn Leu Thr Ala Thr Leu Ser Ser Thr Leu His Gly Lys Glu Ser  
 595 600 605  
 Leu Ile Gly Glu Asp Leu Lys Arg Ala Met Ala Pro Cys Phe Thr Ser  
 610 615 620  
 30 Ala Leu His Leu Thr Ser Gln Glu Val Ala Tyr Asp Leu Leu Leu Trp  
 625 630 635 640  
 35 Ile Asp Gln Ile Gln Pro Ala Gln Ile Thr Val Asp Gly Phe Trp Glu  
 645 650 655  
 Glu Val Gln Thr Thr Pro Thr Ser Leu Lys Val Ile Thr Phe Ala Gln  
 660 665 670  
 40 Val Leu Ala Gln Leu Ser Leu Ile Tyr Arg Arg Ile Gly Leu Ser Glu  
 675 680 685  
 Thr Glu Leu Ser Leu Ile Val Thr Gln Ser Ser Leu Leu Val Ala Gly  
 690 695 700  
 45 Lys Ser Ile Leu Asp His Gly Leu Leu Thr Leu Met Ala Leu Glu Gly  
 705 710 715 720  
 50 Phe His Thr Trp Val Asn Gly Leu Gly Gln His Ala Ser Leu Ile Leu  
 725 730 735  
 Ala Ala Leu Lys Asp Gly Ala Leu Thr Val Thr Asp Val Ala Gln Ala  
 740 745 750  
 55 Met Asn Lys Glu Glu Ser Leu Leu Gln Met Ala Ala Asn Gln Val Glu  
 755 760 765  
 Lys Asp Leu Thr Lys Leu Thr Ser Trp Thr Gln Ile Asp Ala Ile Leu  
 770 775 780  
 60 Gln Trp Leu Gln Met Ser Ser Ala Leu Ala Val Ser Pro Leu Asp Leu  
 785 790 795 800  
 65 Ala Gly Met Met Ala Leu Lys Tyr Gly Ile Asp His Asn Tyr Ala Ala  
 805 810 815 820  
 Trp Gln Ala Ala Ala Ala Ala Leu Met Ala Asp His Ala Asn Gln Ala  
 825 830  
 70 Gln Lys Lys Leu Asp Glu Thr Phe Ser Lys Ala Leu Cys Asn Tyr Tyr  
 835 840 845

	Ile	Asn	Ala	Val	Val	Asp	Ser	Ala	Ala	Gly	Val	Arg	Asp	Arg	Asn	Gly	
		350					855					360					
5	Leu	Tyr	Thr	Tyr	Leu	Leu	Ile	Asp	Asn	Gln	Val	Ser	Ala	Asp	Val	Ile	
	865				370						875					880	
	Thr	Ser	Arg	Ile	Ala	Glu	Ala	Ile	Ala	Gly	Ile	Gln	Leu	Tyr	Val	Asn	
10					885					890					895		
	Arg	Ala	Leu	Asn	Arg	Asp	Glu	Gly	Gln	Leu	Ala	Ser	Asp	Val	Ser	Thr	
				900					905					910			
15	Arg	Gln	Phe	Phe	Thr	Asp	Trp	Glu	Arg	Tyr	Asn	Lys	Arg	Tyr	Ser	Thr	
			915					920					925				
	Trp	Ala	Gly	Val	Ser	Glu	Leu	Val	Tyr	Tyr	Pro	Glu	Asn	Tyr	Val	Asp	
	930						935					940					
20	Pro	Thr	Gln	Arg	Ile	Gly	Gln	Thr	Lys	Met	Met	Asp	Ala	Leu	Leu	Gln	
	945					950					955					960	
	Ser	Ile	Asn	Gln	Ser	Gln	Leu	Asn	Ala	Asp	Thr	Val	Glu	Asp	Ala	Phe	
25					965					970					975		
	Lys	Thr	Tyr	Leu	Thr	Ser	Phe	Glu	Gln	Val	Ala	Asn	Leu	Lys	Val	Ile	
				980					985					990			
30	Ser	Ala	Tyr	His	Asp	Asn	Val	Asn	Val	Asp	Gln	Gly	Leu	Thr	Tyr	Phe	
			995					1000					1005				
	Ile	Gly	Ile	Asp	Gln	Ala	Ala	Pro	Gly	Thr	Tyr	Tyr	Trp	Arg	Ser	Val	
		1010					1015					1020					
35	Asp	His	Ser	Lys	Cys	Glu	Asn	Gly	Lys	Phe	Ala	Ala	Asn	Ala	Trp	Gly	
	1025					1030					1035					1040	
	Glu	Trp	Asn	Lys	Ile	Thr	Cys	Ala	Val	Asn	Pro	Trp	Lys	Asn	Ile	Ile	
40					1045					1050					1055		
	Arg	Pro	Val	Val	Tyr	Met	Ser	Arg	Leu	Tyr	Leu	Leu	Trp	Leu	Glu	Gln	
				1060					1065					1070			
45	Gln	Ser	Lys	Lys	Ser	Asp	Asp	Gly	Lys	Thr	Thr	Ile	Tyr	Gln	Tyr	Asn	
			1075					1080					1085				
	Leu	Lys	Leu	Ala	His	Ile	Arg	Tyr	Asp	Gly	Ser	Trp	Asn	Thr	Pro	Phe	
		1090					1095					1100					
50	Thr	Phe	Asp	Val	Thr	Glu	Lys	Val	Lys	Asn	Tyr	Thr	Ser	Ser	Thr	Asp	
	1105					1110					1115					1120	
	Ala	Ala	Glu	Ser	Leu	Gly	Leu	Tyr	Cys	Thr	Gly	Tyr	Gln	Gly	Glu	Asp	
55					1125					1130					1135		
	Thr	Leu	Leu	Val	Met	Phe	Tyr	Ser	Met	Gln	Ser	Ser	Tyr	Ser	Ser	Tyr	
				1140					1145					1150			
60	Thr	Asp	Asn	Asn	Ala	Pro	Val	Thr	Gly	Leu	Tyr	Ile	Phe	Ala	Asp	Met	
			1155					1160					1165				
	Ser	Ser	Asp	Asn	Met	Thr	Asn	Ala	Gln	Ala	Thr	Asn	Tyr	Trp	Asn	Asn	
			1170				1175					1180					
65	Ser	Tyr	Pro	Gln	Phe	Asp	Thr	Val	Met	Ala	Asp	Pro	Asp	Ser	Asp	Asn	
	1185					1190					1195					1200	
	Lys	Lys	Val	Ile	Thr	Arg	Arg	Val	Asn	Asn	Arg	Tyr	Ala	Glu	Asp	Tyr	
70					1205					1210					1215		
	Glu	Ile	Pro	Ser	Ser	Val	Thr	Ser	Asn	Ser	Asn	Tyr	Ser	Trp	Gly	Asp	

	1220	1225	1230
	His Ser Leu Thr Met Leu Tyr Gly Gly Ser Val Pro Asn Ile Thr Phe 1235 1240 1245		
5	Glu Ser Ala Ala Glu Asp Leu Arg Leu Ser Thr Asn Met Ala Leu Ser 1250 1255 1260		
10	Ile Ile His Asn Gly Tyr Ala Gly Thr Arg Arg Ile Gln Cys Asn Leu 1265 1270 1275 1280		
	Met Lys Gln Tyr Ala Ser Leu Gly Asp Lys Phe Ile Ile Tyr Asp Ser 1285 1290 1295		
15	Ser Phe Asp Asp Ala Asn Arg Phe Asn Leu Val Pro Leu Phe Lys Phe 1300 1305 1310		
	Gly Lys Asp Glu Asn Ser Asp Asp Ser Ile Cys Ile Tyr Asn Glu Asn 1315 1320 1325		
20	Pro Ser Ser Glu Asp Lys Lys Trp Tyr Phe Ser Ser Lys Asp Asp Asn 1330 1335 1340		
25	Lys Thr Ala Asp Tyr Asn Gly Gly Thr Gln Cys Ile Asp Ala Gly Thr 1345 1350 1355 1360		
	Ser Asn Lys Asp Phe Tyr Tyr Asn Leu Gln Glu Ile Glu Val Ile Ser 1365 1370 1375		
30	Val Thr Gly Gly Tyr Trp Ser Ser Tyr Lys Ile Ser Asn Pro Ile Asn 1380 1385 1390		
	Ile Asn Thr Gly Ile Asp Ser Ala Lys Val Lys Val Thr Val Lys Ala 1395 1400 1405		
35	Gly Gly Asp Asp Gln Ile Phe Thr Ala Asp Asn Ser Thr Tyr Val Pro 1410 1415 1420		
40	Gln Gln Pro Ala Pro Ser Phe Glu Glu Met Ile Tyr Gln Phe Asn Asn 1425 1430 1435 1440		
	Leu Thr Ile Asp Cys Lys Asn Leu Asn Phe Ile Asp Asn Gln Ala His 1445 1450 1455		
45	Ile Glu Ile Asp Phe Thr Ala Thr Ala Gln Asp Gly Arg Phe Leu Gly 1460 1465 1470		
	Ala Glu Thr Phe Ile Ile Pro Val Thr Lys Lys Val Leu Gly Thr Glu 1475 1480 1485		
50	Asn Val Ile Ala Leu Tyr Ser Glu Asn Asn Gly Val Gln Tyr Met Gln 1490 1495 1500		
55	Ile Gly Ala Tyr Arg Thr Arg Leu Asn Thr Leu Phe Ala Gln Gln Leu 1505 1510 1515 1520		
	Val Ser Arg Ala Asn Arg Gly Ile Asp Ala Val Leu Ser Met Glu Thr 1525 1530 1535		
60	Gln Asn Ile Gln Glu Pro Gln Leu Gly Ala Gly Thr Tyr Val Gln Leu 1540 1545 1550		
	Val Leu Asp Lys Tyr Asp Glu Ser Ile His Gly Thr Asn Lys Ser Phe 1555 1560 1565		
65	Ala Ile Glu Tyr Val Asp Ile Phe Lys Glu Asn Asp Ser Phe Val Ile 1570 1575 1580		
70	Tyr Gln Gly Glu Leu Ser Glu Thr Ser Gln Thr Val Val Lys Val Phe 1585 1590 1595 1600		

Leu Ser Tyr Phe Ile Glu Ala Thr Gly Asn Lys Asn His Leu Trp Val  
 1605 1610 1615  
 5 Arg Ala Lys Tyr Gln Lys Glu Thr Thr Asp Lys Ile Leu Phe Asp Arg  
 1620 1625 1630  
 Thr Asp Glu Lys Asp Pro His Gly Trp Phe Leu Ser Asp Asp His Lys  
 1635 1640 1645  
 10 Thr Phe Ser Gly Leu Ser Ser Ala Gln Ala Leu Lys Asn Asp Ser Glu  
 1650 1655 1660  
 Pro Met Asp Phe Ser Gly Ala Asn Ala Leu Tyr Phe Trp Glu Leu Phe  
 1665 1670 1675 1680  
 15 Tyr Tyr Thr Pro Met Met Met Ala His Arg Leu Leu Gln Glu Gln Asn  
 1685 1690 1695  
 Phe Asp Ala Ala Asn His Trp Phe Arg Tyr Val Trp Ser Pro Ser Gly  
 1700 1705 1710  
 Tyr Ile Val Asp Gly Lys Ile Ala Ile Tyr His Trp Asn Val Arg Pro  
 1715 1720 1725  
 25 Leu Glu Glu Asp Thr Ser Trp Asn Ala Gln Gln Leu Asp Ser Thr Asp  
 1730 1735 1740  
 Pro Asp Ala Val Ala Gln Asp Asp Pro Met His Tyr Lys Val Ala Thr  
 1745 1750 1755 1760  
 30 Phe Met Ala Thr Leu Asp Leu Leu Met Ala Arg Gly Asp Ala Ala Tyr  
 1765 1770 1775  
 Arg Gln Leu Glu Arg Asp Thr Leu Ala Glu Ala Lys Met Trp Tyr Thr  
 1780 1785 1790  
 Gln Ala Leu Asn Leu Leu Gly Asp Glu Pro Gln Val Met Leu Ser Thr  
 1795 1800 1805  
 40 Thr Trp Ala Asn Pro Thr Leu Gly Asn Ala Ala Ser Lys Thr Thr Gln  
 1810 1815 1820  
 Gln Val Arg Gln Gln Val Leu Thr Gln Leu Arg Leu Asn Ser Arg Val  
 1825 1830 1835 1840  
 45 Lys Thr Pro Leu  
 1844

50 (2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1722 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

60 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:54 (TcbA<sub>III</sub> coding region::

CTA GGA ACA GCC AAT TCC CTG ACC GCT TTA TTC CTG CCG CAG GAA AAT 48  
 Leu Gly Thr Ala Asn Ser Leu Thr Ala Leu Phe Leu Pro Gln Glu Asn  
 1 5 10 15  
 65 AGC AAG CTC AAA GGC TAC TGG CGG ACA CTG GCG CAG CGT ATG TTT AAT 96  
 Ser Lys Leu Lys Gly Tyr Trp Arg Thr Leu Ala Gln Arg Met Phe Asn  
 20 25 30

	TTA CGT CAT AAT CTG TCG ATT GAC GGC CAG CCG CTC TCC TTG CCG CTG 144	
	Leu Arg His Asn Leu Ser Ile Asp Gly Gln Pro Leu Ser Leu Pro Leu	
	35 40 45	
5	TAT GCT AAA CCG GCT GAT CCA AAA GCT TTA CTG AGT GCG GCG GTT TCA 192	
	Tyr Ala Lys Pro Ala Asp Pro Lys Ala Leu Leu Ser Ala Ala Val Ser	
	50 55 60	
10	GCT TCT CAA GGG GGA GCC GAC TTG CCG AAG GCG CCG CTG ACT ATT CAC 240	
	Ala Ser Gln Gly Gly Ala Asp Leu Pro Lys Ala Pro Leu Thr Ile His	
	65 70 75 80	
15	CGC TTC CCT CAA ATG CTA GAA GGG GCA CCG GGC TTG GTT AAC CAG CTT 288	
	Arg Phe Pro Gln Met Leu Glu Gly Ala Arg Gly Leu Val Asn Gln Leu	
	85 90 95	
20	ATA CAG TTC GGT AGT TCA CTA TTG GGG TAC AGT GAG CGT CAG GAT GCG 336	
	Ile Gln Phe Gly Ser Ser Leu Leu Gly Tyr Ser Glu Arg Gln Asp Ala	
	100 105 110	
25	GAA GCT ATG AGT CAA CTA CTG CAA ACC CAA GCC AGC GAG TTA ATA CTG 384	
	Glu Ala Met Ser Gln Leu Leu Thr Gln Ala Ser Glu Leu Ile Leu	
	115 120 125	
30	ACC AGT ATT CGT ATG CAG GAT AAC CAA TTG GCA GAG CTG GAT TCG GAA 432	
	Thr Ser Ile Arg Met Gln Asp Asn Gln Leu Ala Glu Leu Asp Ser Glu	
	130 135 140	
35	AAA ACC GCC TTG CAA GTC TCT TTA GCT GGA GTG CAA CAA CCG TTT GAC 480	
	Lys Thr Ala Leu Gln Val Ser Leu Ala Gly Val Gln Gln Arg Phe Asp	
	145 150 155 160	
40	AGC TAT AGC CAA CTG TAT GAG GAG AAC ATC AAC GCA GGT GAG CAG CGA 528	
	Ser Tyr Ser Gln Leu Tyr Glu Glu Asn Ile Asn Ala Gly Glu Gln Arg	
	165 170 175	
45	GCG CTG GCG TTA CGC TCA GAA TCT GCT ATT GAG TCT CAG GGA GCG CAG 576	
	Ala Leu Ala Leu Arg Ser Glu Ser Ala Ile Glu Ser Gln Gly Ala Gln	
	180 185 190	
50	ATT TCC CGT ATG GCA GGC GCG GGT GTT GAT ATG GCA CCA AAT ATC TTC 624	
	Ile Ser Arg Met Ala Gly Ala Gly Val Asp Met Ala Pro Asn Ile Phe	
	195 200 205	
55	GGC CTG GCT GAT GGC GGC ATG CAT TAT GGT GCT ATT GCC TAT GCC ATC 672	
	Gly Leu Ala Asp Gly Gly Met His Tyr Gly Ala Ile Ala Tyr Ala Ile	
	210 215 220	
60	GCT GAC GGT ATT GAG TTG AGT GCT TCT GCC AAG ATG GTT GAT GCG GAG 720	
	Ala Asp Gly Ile Glu Leu Ser Ala Ser Ala Lys Met Val Asp Ala Glu	
	225 230 235 240	
65	AAA GTT GCT CAG TCG GAA ATA TAT CGC CGT CGC CGT CAA GAA TGG AAA 768	
	Lys Val Ala Gln Ser Glu Ile Tyr Arg Arg Arg Gln Glu Trp Lys	
	245 250 255	
70	ATT CAG CGT GAC AAC GCA CAA GCG GAG ATT AAC CAG TTA AAC GCG CAA 816	
	Ile Gln Arg Asp Asn Ala Gln Ala Glu Ile Asn Gln Leu Asn Ala Gln	
	260 265 270	
75	CTG GAA TCA CTG TCT ATT CGC CGT GAA GCC GCT GAA ATG CAA AAA GAG 864	
	Leu Glu Ser Leu Ser Ile Arg Arg Glu Ala Ala Glu Met Gln Lys Glu	
	275 280 285	
80	TAC CTG AAA ACC CAG CAA GCT CAG GCG CAG GCA CAA CTT ACT TTC TTA 912	
	Tyr Leu Lys Thr Gln Gln Ala Gln Ala Gln Ala Gln Leu Thr Phe Leu	
	290 295 300	
85	AGA AGC AAA TTC AGT AAT CAA GCG TTA TAT AGT TGG TTA CGA GGG CGT 960	
	Arg Ser Lys Phe Ser Asn Gln Ala Leu Tyr Ser Trp Leu Arg Gly Arg	
	305 310 315 320	

	TTG	TCA	GGT	ATT	TAT	TTC	CAG	TTC	TAT	GAC	TTG	GCC	GTA	TCA	CGT	TGC	1008
	Leu	Ser	Gly	Ile	Tyr	Phe	Gln	Phe	Tyr	Asp	Leu	Ala	Val	Ser	Arg	Cys	
					325					330					335		
5	CTG	ATG	GCA	GAG	CAA	TCC	TAT	CAA	TGG	GAA	GCT	AAT	GAT	AAT	TCC	ATT	1056
	Leu	Met	Ala	Glu	Gln	Ser	Tyr	Gln	Trp	Glu	Ala	Asn	Asp	Asn	Ser	Ile	
				340					345					350			
10	AGC	TTT	GTC	AAA	CCG	GGT	GCA	TGG	CAA	GGA	ACT	TAC	GCC	GGC	TTA	TTG	1104
	Ser	Phe	Val	Lys	Pro	Gly	Ala	Trp	Gln	Gly	Thr	Tyr	Ala	Gly	Leu	Leu	
				355				360					365				
15	TGT	GGA	GAA	GCT	TTG	ATA	CAA	AAT	CTG	GCA	CAA	ATG	GAA	GAG	GCA	TAT	1152
	Cys	Gly	Glu	Ala	Leu	Ile	Gln	Asn	Leu	Ala	Gln	Met	Glu	Glu	Ala	Tyr	
		370					375					380					
20	CTG	AAA	TGG	GAA	TCT	CGC	GCT	TTG	GAA	GTA	GAA	CGC	ACG	GTT	TCA	TTG	1200
	Leu	Lys	Trp	Glu	Ser	Arg	Ala	Leu	Glu	Val	Glu	Arg	Thr	Val	Ser	Leu	
		385				390					395					400	
25	GCA	GTG	GTT	TAT	GAT	TCA	CTG	GAA	GGT	AAT	GAT	CGT	TTT	AAT	TTA	GCG	1248
	Ala	Val	Val	Tyr	Asp	Ser	Leu	Glu	Gly	Asn	Asp	Arg	Phe	Asn	Leu	Ala	
					405					410					415		
30	GAA	CAA	ATA	CCT	GCA	TTA	TTG	GAT	AAG	GGG	GAG	GGA	ACA	GCA	GGA	ACT	1296
	Glu	Gln	Ile	Pro	Ala	Leu	Leu	Asp	Lys	Gly	Glu	Gly	Thr	Ala	Gly	Thr	
				420					425					430			
35	AAA	GAA	AAT	GGG	TTA	TCA	TTG	GCT	AAT	GCT	ATC	CTG	TCA	GCT	TCG	GTC	1344
	Lys	Glu	Asn	Gly	Leu	Ser	Leu	Ala	Asn	Ala	Ile	Leu	Ser	Ala	Ser	Val	
				435				440					445				
40	AAA	TTG	TCC	GAC	TTG	AAA	CTG	GGA	ACG	GAT	TAT	CCA	GAC	AGT	ATC	GTT	1392
	Lys	Leu	Ser	Asp	Leu	Lys	Leu	Gly	Thr	Asp	Tyr	Pro	Asp	Ser	Ile	Val	
		450					455					460					
45	GGT	AGC	AAC	AAG	GTT	CGT	CGT	ATT	AAG	CAA	ATC	AGT	GTT	TCG	CTA	CCT	1440
	Gly	Ser	Asn	Lys	Val	Arg	Arg	Ile	Lys	Gln	Ile	Ser	Val	Ser	Leu	Pro	
		465				470					475					480	
50	GCA	TTG	GTT	GGG	CCT	TAT	CAG	GAT	GTT	CAG	GCT	ATG	CTC	AGC	TAT	GGT	1488
	Ala	Leu	Val	Gly	Pro	Tyr	Gln	Asp	Val	Gln	Ala	Met	Leu	Ser	Tyr	Gly	
					485					490					495		
55	GGC	AGT	ACT	CAA	TTG	CCG	AAA	GGT	TGT	TCA	GCG	TTG	GCT	GTG	TCT	CAT	1536
	Gly	Ser	Thr	Gln	Leu	Pro	Lys	Gly	Cys	Ser	Ala	Leu	Ala	Val	Ser	His	
				500					505					510			
60	GGT	ACC	AAT	GAT	AGT	GGT	CAG	TTC	CAG	TTG	GAT	TTC	AAT	GAC	GGC	AAA	1584
	Gly	Thr	Asn	Asp	Ser	Gly	Gln	Phe	Gln	Leu	Asp	Phe	Asn	Asp	Gly	Lys	
				515				520					525				
65	TAC	CTG	CCA	TTT	GAA	GGT	ATT	GCT	CTT	GAT	GAT	CAG	GGT	ACA	CTG	AAT	1632
	Tyr	Leu	Pro	Phe	Glu	Gly	Ile	Ala	Leu	Asp	Asp	Gln	Gly	Thr	Leu	Asn	
		530					535					540					
70	CTT	CAA	TTT	CCG	AAT	GCT	ACC	GAC	AAG	CAG	AAA	GCA	ATA	TTG	CAA	ACT	1680
	Leu	Gln	Phe	Pro	Asn	Ala	Thr	Asp	Lys	Gln	Lys	Ala	Ile	Leu	Gln	Thr	
		545				550					555					560	
	ATG	AGC	GAT	ATT	ATT	TTG	CAT	ATT	CGT	TAT	ACC	ATC	CGT	TAA			1722
	Met	Ser	Asp	Ile	Ile	Leu	His	Ile	Arg	Tyr	Thr	Ile	Arg	...			
					565				570				573				

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 573 amino acids

(B) TYPE: amino acids

(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55 (TcbAiii):

10	Leu	Gly	Thr	Ala	Asn	Ser	Leu	Thr	Ala	Leu	Phe	Leu	Pro	Gln	Glu	Asn	1	5	10	15
	Ser	Lys	Leu	Lys	Gly	Tyr	Trp	Arg	Thr	Leu	Ala	Gln	Arg	Met	Phe	Asn	20	25	30	
15	Leu	Arg	His	Asn	Leu	Ser	Ile	Asp	Gly	Gln	Pro	Leu	Ser	Leu	Pro	Leu	35	40	45	
	Tyr	Ala	Lys	Pro	Ala	Asp	Pro	Lys	Ala	Leu	Leu	Ser	Ala	Ala	Val	Ser	50	55	60	
20	Ala	Ser	Gln	Gly	Gly	Ala	Asp	Leu	Pro	Lys	Ala	Pro	Leu	Thr	Ile	His	65	70	75	80
	Arg	Phe	Pro	Gln	Met	Leu	Glu	Gly	Ala	Arg	Gly	Leu	Val	Asn	Gln	Leu	85	90	95	
25	Ile	Gln	Phe	Gly	Ser	Ser	Leu	Leu	Gly	Tyr	Ser	Glu	Arg	Gln	Asp	Ala	100	105	110	
	Glu	Ala	Met	Ser	Gln	Leu	Leu	Gln	Thr	Gln	Ala	Ser	Glu	Leu	Ile	Leu	115	120	125	
30	Thr	Ser	Ile	Arg	Met	Gln	Asp	Asn	Gln	Leu	Ala	Glu	Leu	Asp	Ser	Glu	130	135	140	
35	Lys	Thr	Ala	Leu	Gln	Val	Ser	Leu	Ala	Gly	Val	Gln	Gln	Arg	Phe	Asp	145	150	155	160
	Ser	Tyr	Ser	Gln	Leu	Tyr	Glu	Glu	Asn	Ile	Asn	Ala	Gly	Glu	Gln	Arg	165	170	175	
40	Ala	Leu	Ala	Leu	Arg	Ser	Glu	Ser	Ala	Ile	Glu	Ser	Gln	Gly	Ala	Gln	180	185	190	
	Ile	Ser	Arg	Met	Ala	Gly	Ala	Gly	Val	Asp	Met	Ala	Pro	Asn	Ile	Phe	195	200	205	
45	Gly	Leu	Ala	Asp	Gly	Gly	Met	His	Tyr	Gly	Ala	Ile	Ala	Tyr	Ala	Ile	210	215	220	
50	Ala	Asp	Gly	Ile	Glu	Leu	Ser	Ala	Ser	Ala	Lys	Met	Val	Asp	Ala	Glu	225	230	235	240
	Lys	Val	Ala	Gln	Ser	Glu	Ile	Tyr	Arg	Arg	Arg	Arg	Gln	Glu	Trp	Lys	245	250	255	
55	Ile	Gln	Arg	Asp	Asn	Ala	Gln	Ala	Glu	Ile	Asn	Gln	Leu	Asn	Ala	Gln	260	265	270	
	Leu	Glu	Ser	Leu	Ser	Ile	Arg	Arg	Glu	Ala	Ala	Glu	Met	Gln	Lys	Glu	275	280	285	
60	Tyr	Leu	Lys	Thr	Gln	Gln	Ala	Gln	Ala	Gln	Ala	Gln	Leu	Thr	Phe	Leu	290	295	300	
65	Arg	Ser	Lys	Phe	Ser	Asn	Gln	Ala	Leu	Tyr	Ser	Trp	Leu	Arg	Gly	Arg	305	310	315	320
	Leu	Ser	Gly	Ile	Tyr	Phe	Gln	Phe	Tyr	Asp	Leu	Ala	Val	Ser	Arg	Cys	325	330	335	
70																				

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SUBSTITUTE SHEET (RULE 26)

Leu Met Ala Glu Gln Ser Tyr Gln Trp Glu Ala Asn Asp Asn Ser Ile  
                   340                  345                  350  
 5 Ser Phe Val Lys Pro Gly Ala Trp Gln Gly Thr Tyr Ala Gly Leu Leu  
                   355                  360                  365  
 Cys Gly Glu Ala Leu Ile Gln Asn Leu Ala Gln Met Glu Glu Ala Tyr  
                   370                  375                  380  
 10 Leu Lys Trp Glu Ser Arg Ala Leu Glu Val Glu Arg Thr Val Ser Leu  
                   385                  390                  395                  400  
 Ala Val Val Tyr Asp Ser Leu Glu Gly Asn Asp Arg Phe Asn Leu Ala  
                   405                  410                  415  
 15 Glu Gln Ile Pro Ala Leu Leu Asp Lys Gly Glu Gly Thr Ala Gly Thr  
                   420                  425                  430  
 20 Lys Glu Asn Gly Leu Ser Leu Ala Asn Ala Ile Leu Ser Ala Ser Val  
                   435                  440                  445  
 Lys Leu Ser Asp Leu Lys Leu Gly Thr Asp Tyr Pro Asp Ser Ile Val  
                   450                  455                  460  
 25 Gly Ser Asn Lys Val Arg Arg Ile Lys Gln Ile Ser Val Ser Leu Pro  
                   465                  470                  475                  480  
 Ala Leu Val Gly Pro Tyr Gln Asp Val Gln Ala Met Leu Ser Tyr Gly  
                   485                  490                  495  
 30 Gly Ser Thr Gln Leu Pro Lys Gly Cys Ser Ala Leu Ala Val Ser His  
                   500                  505                  510  
 35 Gly Thr Asn Asp Ser Gly Gln Phe Gln Leu Asp Phe Asn Asp Gly Lys  
                   515                  520                  525  
 Tyr Leu Pro Phe Glu Gly Ile Ala Leu Asp Asp Gln Gly Thr Leu Asn  
                   530                  535                  540  
 40 Leu Gln Phe Pro Asn Ala Thr Asp Lys Gln Lys Ala Ile Leu Gln Thr  
                   545                  550                  555                  560  
 45 Met Ser Asp Ile Ile Leu His Ile Arg Tyr Thr Ile Arg ...  
                   565                  570                  573

## (2) INFORMATION FOR SEQ ID NO:56

(i) SEQUENCE CHARACTERISTICS:  
 50 (A) LENGTH: 2898 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

55 (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:56 (tcca)

60 1 ATG AAT CAA CTC GCC AGT CCC CTG ATT TCC CGC ACC GAA GAG ATC CAC 48  
 1 Met Asn Gln Leu Ala Ser Pro Leu Ile Ser Arg Thr Glu Glu Ile His 16  
 49 AAC TTA CCC GGT AAA TTG ACC GAT CTT GGT TAT ACC TCA GTG TTT GAT 96  
 65 17 Asn Leu Pro Gly Lys Leu Thr Asp Leu Gly Tyr Thr Ser Val Phe Asp 32  
 97 GTG GTA CGT ATG CCG CGT GAG CGT TTT ATT CGT GAG CAT CGT GCT GAT 144



	33	Val	Val	Arg	Met	Pro	Arg	Glu	Arg	Phe	Ile	Arg	Glu	His	Arg	Ala	Asp	43
5	145	CTC	GGG	CGC	AGT	GCT	GAA	AAA	ATG	TAT	GAC	CTG	GCA	GTG	GGC	TAT	GCT	192
	49	Leu	Gly	Arg	Ser	Ala	Glu	Lys	Met	Tyr	Asp	Leu	Ala	Val	Gly	Tyr	Ala	64
10	193	CAT	CAG	GTG	TTA	CAC	CAT	TTT	CGC	CGT	AAT	TCT	CTT	AGT	GAA	GCT	GTT	240
	65	His	Gln	Val	Leu	His	His	Phe	Arg	Arg	Asn	Ser	Leu	Ser	Glu	Ala	Val	30
15	241	CAG	TTT	GGC	TTG	AGA	AGT	CCG	TTC	TCC	GTA	TCA	GGC	CCG	GAT	TAC	GCC	288
	81	Gln	Phe	Gly	Leu	Arg	Ser	Pro	Phe	Ser	Val	Ser	Gly	Pro	Asp	Tyr	Ala	96
20	289	AAT	CAG	TTT	CTT	GAT	GCA	AAC	ACG	GGT	TGG	AAA	GAT	AAA	GCA	CCA	AGT	336
	97	Asn	Gln	Phe	Leu	Asp	Ala	Asn	Thr	Gly	Trp	Lys	Asp	Lys	Ala	Pro	Ser	112
25	337	GGA	TCA	CCG	GAA	GCC	AAT	GAT	GCG	CCG	GTA	GCC	TAT	CTG	ACT	CAT	ATT	384
	113	Gly	Ser	Pro	Glu	Ala	Asn	Asp	Ala	Pro	Val	Ala	Tyr	Leu	Thr	His	Ile	128
30	385	TAT	CAA	TTG	GCC	CTT	GAA	CAG	GAA	AAG	AAT	GGC	GCC	ACT	ACC	ATT	ATG	432
	129	Tyr	Gln	Leu	Ala	Leu	Glu	Gln	Glu	Lys	Asn	Gly	Ala	Thr	Thr	Ile	Met	144
35	433	AAT	ACG	CTG	GCG	GAG	CGT	CGC	CCC	GAT	CTG	GGT	GCT	TTG	TTA	ATT	AAT	480
	145	Asn	Thr	Leu	Ala	Glu	Arg	Arg	Pro	Asp	Leu	Gly	Ala	Leu	Leu	Ile	Asn	160
40	481	GAT	AAA	GCA	ATC	AAT	GAG	GTG	ATA	CCG	CAA	TTG	CAG	TTG	GTC	AAT	GAA	528
	161	Asp	Lys	Ala	Ile	Asn	Glu	Val	Ile	Pro	Gln	Leu	Gln	Leu	Val	Asn	Glu	176
45	529	ATT	CTG	TCC	AAA	GCT	ATT	CAG	AAG	AAA	CTG	AGT	TTG	ACT	GAT	CTG	GAA	576
	177	Ile	Leu	Ser	Lys	Ala	Ile	Gln	Lys	Lys	Leu	Ser	Leu	Thr	Asp	Leu	Glu	192
50	577	GCG	GTA	AAC	GCC	AGA	CTT	TCC	ACT	ACC	CGT	TAC	CCG	AAT	AAT	CTG	CCG	624
	193	Ala	Val	Asn	Ala	Arg	Leu	Ser	Thr	Thr	Arg	Tyr	Pro	Asn	Asn	Leu	Pro	208
55	625	TAT	CAT	TAT	GGT	CAT	CAG	CAG	ATT	CAG	ACA	GCT	CAA	TCG	GTA	TTG	GGT	672
	209	Tyr	His	Tyr	Gly	His	Gln	Gln	Ile	Gln	Thr	Ala	Gln	Ser	Val	Leu	Gly	224
60	673	ACT	ACG	TTG	CAA	GAT	ATC	ACT	TTG	CCA	CAG	ACG	CTG	GAT	CTG	CCG	CAA	720
	225	Thr	Thr	Leu	Gln	Asp	Ile	Thr	Leu	Pro	Gln	Thr	Leu	Asp	Leu	Pro	Gln	240
65	721	AAC	TTC	TGG	GCA	ACA	GCA	AAA	GGA	AAA	CTG	AGC	GAT	ACG	ACT	GCC	AGT	768
	241	Asn	Phe	Trp	Ala	Thr	Ala	Lys	Gly	Lys	Leu	Ser	Asp	Thr	Thr	Ala	Ser	256
60	769	GCT	TTG	ACC	CGA	CTG	CAA	ATC	ATG	GCG	AGT	CAG	TTT	TCG	CCA	GAG	CAG	816
	257	Ala	Leu	Thr	Arg	Leu	Gln	Ile	Met	Ala	Ser	Gln	Phe	Ser	Pro	Glu	Gln	272
65	817	CAG	AAA	ATC	ATT	ACG	GAG	ACT	GTC	GGT	CAG	GAT	TTC	TAT	CAG	CTT	AAC	864
	273	Gln	Lys	Ile	Ile	Thr	Glu	Thr	Val	Gly	Gln	Asp	Phe	Tyr	Gln	Leu	Asn	288
65	865	TAT	GGT	GAC	AGT	TCG	CTT	ACT	GTG	AAT	AGT	TTC	AGC	GAC	ATG	ACC	ATA	912
	289	Tyr	Gly	Asp	Ser	Ser	Leu	Thr	Val	Asn	Ser	Phe	Ser	Asp	Met	Thr	Ile	304

	313	ATG ACT GAT CGA ACA AGT TTG ACT GTA CCC CAG GTA GAA CTG ATG TTG	360
	305	Met Thr Asp Arg Thr Ser Leu Thr Val Pro Gln Val Glu Leu Met Leu	310
5			
	951	TGT TCA ACT GTC GGA GGT TCT ACG GTT GTT AAG TCT GAT AAT GTG AGT	1003
	321	Cys Ser Thr Val Gly Gly Ser Thr Val Val Lys Ser Asp Asn Val Ser	335
10			
	1009	TCT GGT GAC ACG ACA GCG ACG CCA TTT GCG TAT GGC GCC CGC TTT ATT	1056
	337	Ser Gly Asp Thr Thr Ala Thr Pro Phe Ala Tyr Gly Ala Arg Phe Ile	352
15			
	1057	CAT GCC GGT AAG CCG GAG GCG ATT ACC CTG AGT CGC AGT GGT GCG GAG	1104
	353	His Ala Gly Lys Pro Glu Ala Ile Thr Leu Ser Arg Ser Gly Ala Glu	368
20			
	1105	GCG CAT TTT GCT CTG ACG GTT AAC AAT CTG ACA GAT GAC AAG TTG GAC	1152
	369	Ala His Phe Ala Leu Thr Val Asn Asn Leu Thr Asp Asp Lys Leu Asp	384
	1153	CGT ATT AAC CGC ACA GTG CGC CTG CAA AAA TGG CTG AAT CTG CCT TAT	1200
	385	Arg Ile Asn Arg Thr Val Arg Leu Gln Lys Trp Leu Asn Leu Pro Tyr	400
25			
	1201	GAG GAT ATT GAC CTG TTA GTG ACT TCT GCT ATG GAT GCG GAA ACA GGA	1248
	401	Glu Asp Ile Asp Leu Leu Val Thr Ser Ala Met Asp Ala Glu Thr Gly	416
30			
	1249	AAT ACC GCG CTG TCG ATG AAC GAC AAT ACG CTG CGT ATG TTG GGA GTG	1296
	417	Asn Thr Ala Leu Ser Met Asn Asp Asn Thr Leu Arg Met Leu Gly Val	432
35			
	1297	TTC AAA CAT TAT CAG GCG AAG TAT GGT GTT AGC GCT AAA CAA TTT GCT	1344
	433	Phe Lys His Tyr Gln Ala Lys Tyr Gly Val Ser Ala Lys Gln Phe Ala	448
40			
	1345	GGC TGG CTG CGC GTA GTG GCC CCG TTT GCC ATT ACA CCG GCA ACG CCG	1392
	449	Gly Trp Leu Arg Val Val Ala Pro Phe Ala Ile Thr Pro Ala Thr Pro	464
	1393	TTT TTA GAC CAA GTG TTT AAC TCC GTC GGC ACC TTT GAT ACA CCG TTT	1440
	465	Phe Leu Asp Gln Val Phe Asn Ser Val Gly Thr Phe Asp Thr Pro Phe	480
45			
	1441	GTG ATA GAT AAT CAG GAT TTT GTC TAT ACA TTG ACC ACC GGG GGC GAT	1488
	481	Val Ile Asp Asn Gln Asp Phe Val Tyr Thr Leu Thr Thr Gly Gly Asp	496
50			
	1489	GGG GCG CGT GTT AAG CAT ATC AGC ACG GCA CTG GGC CTC AAT CAT CGT	1536
	497	Gly Ala Arg Val Lys His Ile Ser Thr Ala Leu Gly Leu Asn His Arg	512
55			
	1537	CAG TTC CTG TTA TTG GCG GAT AAT ATT GCC CGT CAA CAG GGG AAT GTC	1584
	513	Gln Phe Leu Leu Leu Ala Asp Asn Ile Ala Arg Gln Gln Gly Asn Val	528
60			
	1585	ACG CAA AGC ACA CTC AAC TGT AAT CTG TTT GTG GTG TCA GCT TTC TAC	1632
	529	Thr Gln Ser Thr Leu Asn Cys Asn Leu Phe Val Val Ser Ala Phe Tyr	544
	1633	CGT CTG GCT AAT TTG GCG CGC ACA TTG GGG ATA AAT CCA GAG TCT TTC	1680
	545	Arg Leu Ala Asn Leu Ala Arg Thr Leu Gly Ile Asn Pro Glu Ser Phe	560
65			
	1681	TGT GCC TTG GTT GAT CGA TTA GAT GCA GGT ACA GGC ATC GTC TGG CAG	1728

	561	Cys	Ala	Leu	Val	Asp	Arg	Leu	Asp	Ala	Gly	Thr	Gly	Ile	Val	Trp	Gln	574
5	1729	CAA	TTG	GCA	GGG	AAA	CCC	ACA	ATC	ACG	GTA	CCA	CAA	AAA	GAT	TCC	CCG	1776
	577	Gln	Leu	Ala	Gly	Lys	Pro	Thr	Ile	Thr	Val	Pro	Gln	Lys	Asp	Ser	Pro	590
10	1777	CTG	GCG	GCG	GAT	ATT	CTG	AGT	TTG	CTG	CAA	GCG	CTA	AGT	GCG	ATT	GCT	1824
	593	Leu	Ala	Ala	Asp	Ile	Leu	Ser	Leu	Leu	Gln	Ala	Leu	Ser	Ala	Ile	Ala	608
15	1825	CAA	TGG	CAA	CAA	CAG	CAC	GAT	TTA	GAA	TTT	TCA	GCA	CTG	CTT	TTG	CTG	1872
	609	Gln	Trp	Gln	Gln	Gln	His	Asp	Leu	Glu	Phe	Ser	Ala	Leu	Leu	Leu	Leu	624
20	1873	TTG	AGT	GAC	AAC	CCT	ATT	TCT	ACC	TCG	CAG	GGC	ACT	GAC	GAT	CAA	TTG	1920
	625	Leu	Ser	Asp	Asn	Pro	Ile	Ser	Thr	Ser	Gln	Gly	Thr	Asp	Asp	Gln	Leu	640
25	1921	AAC	TTT	ATC	CGT	CAA	GTG	TGG	CAG	AAC	CTA	GGC	AGT	ACG	TTT	GTG	GGT	1968
	641	Asn	Phe	Ile	Arg	Gln	Val	Trp	Gln	Asn	Leu	Gly	Ser	Thr	Phe	Val	Gly	656
30	1969	GCA	ACA	TTG	TTG	TCC	CGC	AGT	GGG	GCA	CCA	TTA	GTC	GAT	ACC	AAC	GGC	2016
	657	Ala	Thr	Leu	Leu	Ser	Arg	Ser	Gly	Ala	Pro	Leu	Val	Asp	Thr	Asn	Gly	672
35	2017	CAC	GCT	ATT	GAC	TGG	TTT	GCT	CTG	CTC	TCA	GCA	GGT	AAT	AGT	CCG	CTT	2064
	673	His	Ala	Ile	Asp	Trp	Phe	Ala	Leu	Leu	Ser	Ala	Gly	Asn	Ser	Pro	Leu	688
40	2065	ATC	GAT	AAG	GTT	GGT	CTG	GTG	ACT	GAT	GCT	GGC	ATA	CAA	AGT	GTT	ATA	2112
	689	Ile	Asp	Lys	Val	Gly	Leu	Val	Thr	Asp	Ala	Gly	Ile	Gln	Ser	Val	Ile	704
45	2113	GCA	ACG	GTG	GTC	AAT	ACA	CAA	AGC	TTA	TCT	GAT	GAA	GAT	AAG	AAG	CTG	2160
	705	Ala	Thr	Val	Val	Asn	Thr	Gln	Ser	Leu	Ser	Asp	Glu	Asp	Lys	Lys	Leu	720
50	2161	GCA	ATC	ACT	ACT	CTG	ACT	AAT	ACG	TTG	AAT	CAG	GTA	CAG	AAA	ACT	CAA	2208
	721	Ala	Ile	Thr	Thr	Leu	Thr	Asn	Thr	Leu	Asn	Gln	Val	Gln	Lys	Thr	Gln	736
55	2209	CAG	GGC	GTG	GCC	GTC	AGT	CTG	TTG	GCG	CAG	ACT	CTG	AAC	GTG	AGT	CAG	2256
	737	Gln	Gly	Val	Ala	Val	Ser	Leu	Leu	Ala	Gln	Thr	Leu	Asn	Val	Ser	Gln	752
60	2257	TCA	CTG	CCT	GCG	TTA	TTG	TTG	CGC	TGG	AGT	GGA	CAA	ACA	ACC	TAC	CAG	2304
	753	Ser	Leu	Pro	Ala	Leu	Leu	Leu	Arg	Trp	Ser	Gly	Gln	Thr	Thr	Tyr	Gln	768
65	2305	TGG	TTG	AGT	GCG	ACT	TGG	GCA	TTG	AAG	GAT	GCC	GTT	AAG	ACT	GCC	GCC	2352
	769	Trp	Leu	Ser	Ala	Thr	Trp	Ala	Leu	Lys	Asp	Ala	Val	Lys	Thr	Ala	Ala	784
	2353	GAT	ATT	CCC	GCT	GAC	TAT	CTG	CGT	CAA	TTA	CGT	GAA	GTG	GTA	CGC	CGC	2400
	785	Asp	Ile	Pro	Ala	Asp	Tyr	Leu	Arg	Gln	Leu	Arg	Glu	Val	Val	Arg	Arg	800
	2401	TCC	TTG	TTG	ACC	CAA	CAA	TTC	ACG	CTG	AGT	CCT	GCA	ATG	GTG	CAA	ACC	2448
	801	Ser	Leu	Leu	Thr	Gln	Gln	Phe	Thr	Leu	Ser	Pro	Ala	Met	Val	Gln	Thr	816

	2443	TTG CTG GAC TAT CCA GCC TAT TTT GGC GCT TCC GCA GAA ACA GTG ACC	2496
	317	Leu Leu Asp Tyr Pro Ala Tyr Phe Gly Ala Ser Ala Glu Thr Val Thr	332
5	2497	GAT ATC AGT TTG TGG ATG CTT TAT ACC CTG AGC TGT TAT AGC GAT TTA	2544
	333	Asp Ile Ser Leu Trp Met Leu Tyr Thr Leu Ser Cys Tyr Ser Asp Leu	348
10	2545	TTG CTC CAA ATG GGT GAA GCT GGT GGT ACC GAA GAT GAT GTA CTG GCC	2592
	349	Leu Leu Gln Met Gly Glu Ala Gly Gly Thr Glu Asp Asp Val Leu Ala	364
15	2593	TAC TTA CGC ACA GCT AAT GCT ACC ACA CCG TTG AGC CAA TCT GAT GCT	2640
	365	Tyr Leu Arg Thr Ala Asn Ala Thr Thr Pro Leu Ser Gln Ser Asp Ala	380
20	2641	GCA CAG ACG TTG GCA ACG CTA TTG GGT TGG GAG GTT AAC GAG TTG CAA	2688
	381	Ala Gln Thr Leu Ala Thr Leu Leu Gly Trp Glu Val Asn Glu Leu Gln	396
	2689	GCC GCT TGG TCG GTA TTG GGC GGG ATT GCC AAA ACC ACA CCG CAA CTG	2736
	397	Ala Ala Trp Ser Val Leu Gly Gly Ile Ala Lys Thr Thr Pro Gln Leu	912
25	2737	GAT GCG CTT CTG CGT TTG CAA CAG GCA CAG AAC CAA ACT GGT CTT GGC	2784
	913	Asp Ala Leu Leu Arg Leu Gln Gln Ala Gln Asn Gln Thr Gly Leu Gly	928
30	2785	GTT ACA CAG CAA CAG CAA GGC TAT CTC CTG AGT CGT GAC AGT GAT TAT	2832
	929	Val Thr Gln Gln Gln Gln Gly Tyr Leu Leu Ser Arg Asp Ser Asp Tyr	944
35	2833	ACC CTT TGG CAA AGC ACC GGT CAG GCG CTG GTG GCT GGC GTA TCC CAT	2880
	945	Thr Leu Trp Gln Ser Thr Gly Gln Ala Leu Val Ala Gly Val Ser His	960
	2881	GTC AAG GGC AGT AAC TGA	2898
	961	Val Lys Gly Ser Asn End	966
40	(2)	INFORMATION FOR SEQ ID NO:57	
	(i)	SEQUENCE CHARACTERISTICS:	
		(A)	LENGTH: 965 amino acids
		(B)	TYPE: amino acid
45		(C)	TOPOLOGY: linear
	(ii)	MOLECULE TYPE: protein	
50	(xi)	SEQUENCE DESCRIPTION: SEQ ID NO:57 (TCCA peptide)	
	Features	From To Description	
		1 10	SEQ ID NO:8
55	1	Met Asn Gln Leu Ala Ser Pro Leu Ile Ser Arg Thr Glu Glu Ile His	16
	17	Asn Leu Pro Gly Lys Leu Thr Asp Leu Gly Tyr Thr Ser Val Phe Asp	32
	33	Val Val Arg Met Pro Arg Glu Arg Phe Ile Arg Glu His Arg Ala Asp	48
60	49	Leu Gly Arg Ser Ala Glu Lys Met Tyr Asp Leu Ala Val Gly Tyr Ala	64
	65	His Gln Val Leu His His Phe Arg Arg Asn Ser Leu Ser Glu Ala Val	80
65	81	Gln Phe Gly Leu Arg Ser Pro Phe Ser Val Ser Gly Pro Asp Tyr Ala	96

	97	Asn	Gln	Phe	Leu	Asp	Ala	Asn	Thr	Gly	Trp	Lys	Asp	Lys	Ala	Pro	Ser	111
	113	Gly	Ser	Pro	Glu	Ala	Asn	Asp	Ala	Pro	Val	Ala	Tyr	Leu	Thr	His	Ile	128
5	129	Tyr	Gln	Leu	Ala	Leu	Glu	Gln	Glu	Lys	Asn	Gly	Ala	Thr	Thr	Ile	Met	144
	145	Asn	Thr	Leu	Ala	Glu	Arg	Arg	Pro	Asp	Leu	Gly	Ala	Leu	Leu	Ile	Asn	160
	161	Asp	Lys	Ala	Ile	Asn	Glu	Val	Ile	Pro	Gln	Leu	Gln	Leu	Val	Asn	Glu	176
10	177	Ile	Leu	Ser	Lys	Ala	Ile	Gln	Lys	Lys	Leu	Ser	Leu	Thr	Asp	Leu	Glu	192
	193	Ala	Val	Asn	Ala	Arg	Leu	Ser	Thr	Thr	Arg	Tyr	Pro	Asn	Asn	Leu	Pro	208
15	209	Tyr	His	Tyr	Gly	His	Gln	Gln	Ile	Gln	Thr	Ala	Gln	Ser	Val	Leu	Gly	224
	225	Thr	Thr	Leu	Gln	Asp	Ile	Thr	Leu	Pro	Gln	Thr	Leu	Asp	Leu	Pro	Gln	240
	241	Asn	Phe	Trp	Ala	Thr	Ala	Lys	Gly	Lys	Leu	Ser	Asp	Thr	Thr	Ala	Ser	256
20	257	Ala	Leu	Thr	Arg	Leu	Gln	Ile	Met	Ala	Ser	Gln	Phe	Ser	Pro	Glu	Gln	272
	273	Gln	Lys	Ile	Ile	Thr	Glu	Thr	Val	Gly	Gln	Asp	Phe	Tyr	Gln	Leu	Asn	288
25	289	Tyr	Gly	Asp	Ser	Ser	Leu	Thr	Val	Asn	Ser	Phe	Ser	Asp	Met	Thr	Ile	304
	305	Met	Thr	Asp	Arg	Thr	Ser	Leu	Thr	Val	Pro	Gln	Val	Glu	Leu	Met	Leu	320
	321	Cys	Ser	Thr	Val	Gly	Gly	Ser	Thr	Val	Val	Lys	Ser	Asp	Asn	Val	Ser	336
30	337	Ser	Gly	Asp	Thr	Thr	Ala	Thr	Pro	Phe	Ala	Tyr	Gly	Ala	Arg	Phe	Ile	352
	353	His	Ala	Gly	Lys	Pro	Glu	Ala	Ile	Thr	Leu	Ser	Arg	Ser	Gly	Ala	Glu	368
35	369	Ala	His	Phe	Ala	Leu	Thr	Val	Asn	Asn	Leu	Thr	Asp	Asp	Lys	Leu	Asp	384
	385	Arg	Ile	Asn	Arg	Thr	Val	Arg	Leu	Gln	Lys	Trp	Leu	Asn	Leu	Pro	Tyr	400
	401	Glu	Asp	Ile	Asp	Leu	Leu	Val	Thr	Ser	Ala	Met	Asp	Ala	Glu	Thr	Gly	416
40	417	Asn	Thr	Ala	Leu	Ser	Met	Asn	Asp	Asn	Thr	Leu	Arg	Met	Leu	Gly	Val	432
	433	Phe	Lys	His	Tyr	Gln	Ala	Lys	Tyr	Gly	Val	Ser	Ala	Lys	Gln	Phe	Ala	448
45	449	Gly	Trp	Leu	Arg	Val	Val	Ala	Pro	Phe	Ala	Ile	Thr	Pro	Ala	Thr	Pro	464
	465	Phe	Leu	Asp	Gln	Val	Phe	Asn	Ser	Val	Gly	Thr	Phe	Asp	Thr	Pro	Phe	480
	481	Val	Ile	Asp	Asn	Gln	Asp	Phe	Val	Tyr	Thr	Leu	Thr	Thr	Gly	Gly	Asp	496
50	497	Gly	Ala	Arg	Val	Lys	His	Ile	Ser	Thr	Ala	Leu	Gly	Leu	Asn	His	Arg	512
	513	Gln	Phe	Leu	Leu	Leu	Ala	Asp	Asn	Ile	Ala	Arg	Gln	Gln	Gly	Asn	Val	528
55	529	Thr	Gln	Ser	Thr	Leu	Asn	Cys	Asn	Leu	Phe	Val	Val	Ser	Ala	Phe	Tyr	544
	545	Arg	Leu	Ala	Asn	Leu	Ala	Arg	Thr	Leu	Gly	Ile	Asn	Pro	Glu	Ser	Phe	560
	561	Cys	Ala	Leu	Val	Asp	Arg	Leu	Asp	Ala	Gly	Thr	Gly	Ile	Val	Trp	Gln	576
60	577	Gln	Leu	Ala	Gly	Lys	Pro	Thr	Ile	Thr	Val	Pro	Gln	Lys	Asp	Ser	Pro	592
	593	Leu	Ala	Ala	Asp	Ile	Leu	Ser	Leu	Leu	Gln	Ala	Leu	Ser	Ala	Ile	Ala	608
65	609	Gln	Trp	Gln	Gln	Gln	His	Asp	Leu	Glu	Phe	Ser	Ala	Leu	Leu	Leu	Leu	624

625 Leu Ser Asp Asn Pro Ile Ser Thr Ser Gln Gly Thr Asp Asp Gln Leu 640  
 641 Asn Phe Ile Arg Gln Val Trp Gln Asn Leu Gly Ser Thr Phe Val Gly 655  
 5 657 Ala Thr Leu Leu Ser Arg Ser Gly Ala Pro Leu Val Asp Thr Asn Gly 672  
 673 His Ala Ile Asp Trp Phe Ala Leu Leu Ser Ala Gly Asn Ser Pro Leu 688  
 10 689 Ile Asp Lys Val Gly Leu Val Thr Asp Ala Gly Ile Gln Ser Val Ile 704  
 705 Ala Thr Val Val Asn Thr Gln Ser Leu Ser Asp Glu Asp Lys Lys Leu 720  
 721 Ala Ile Thr Thr Leu Thr Asn Thr Leu Asn Gln Val Gln Lys Thr Gln 736  
 15 737 Gln Gly Val Ala Val Ser Leu Leu Ala Gln Thr Leu Asn Val Ser Gln 752  
 753 Ser Leu Pro Ala Leu Leu Leu Arg Trp Ser Gly Gln Thr Thr Tyr Gln 768  
 20 769 Trp Leu Ser Ala Thr Trp Ala Leu Lys Asp Ala Val Lys Thr Ala Ala 784  
 785 Asp Ile Pro Ala Asp Tyr Leu Arg Gln Leu Arg Glu Val Val Arg Arg 800  
 801 Ser Leu Leu Thr Gln Gln Phe Thr Leu Ser Pro Ala Met Val Gln Thr 816  
 25 817 Leu Leu Asp Tyr Pro Ala Tyr Phe Gly Ala Ser Ala Glu Thr Val Thr 832  
 833 Asp Ile Ser Leu Trp Met Leu Tyr Thr Leu Ser Cys Tyr Ser Asp Leu 848  
 30 849 Leu Leu Gln Met Gly Glu Ala Gly Gly Thr Glu Asp Asp Val Leu Ala 864  
 865 Tyr Leu Arg Thr Ala Asn Ala Thr Thr Pro Leu Ser Gln Ser Asp Ala 880  
 881 Ala Gln Thr Leu Ala Thr Leu Leu Gly Trp Glu Val Asn Glu Leu Gln 896  
 35 897 Ala Ala Trp Ser Val Leu Gly Gly Ile Ala Lys Thr Thr Pro Gln Leu 912  
 913 Asp Ala Leu Leu Arg Leu Gln Gln Ala Gln Asn Gln Thr Gly Leu Gly 928  
 40 929 Val Thr Gln Gln Gln Gln Gly Tyr Leu Leu Ser Arg Asp Ser Asp Tyr 944  
 945 Thr Leu Trp Gln Ser Thr Gly Gln Ala Leu Val Ala Gly Val Ser His 960  
 961 Val Lys Gly Ser Asn 965

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## (2) INFORMATION FOR SEQ ID NO:58

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4698 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

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## (ii) MOLECULE TYPE: DNA (genomic)

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:58 (tccB)

1 ATG TTA TCG ACA ATG GAA AAA CAA CTG AAT GAA TCC CAG CGT GAT GCG 43  
 1 Met Leu Ser Thr Met Glu Lys Gln Leu Asn Glu Ser Gln Arg Asp Ala 16  
 49 TTG GTG ACT GGC TAT ATG AAT TTT GTG GCG CCG ACG TTG AAA GGC GTC 96  
 17 Leu Val Thr Gly Tyr Met Asn Phe Val Ala Pro Thr Leu Lys Gly Val 32

65

	97	AGT GGT CAG CCG GTG ACG GTG GAA GAT TTA TAC GAA TAT TTG CTG ATT	144
	33	Ser Gly Gln Pro Val Thr Val Glu Asp Leu Tyr Glu Tyr Leu Leu Ile	48
5	145	GAC CCG GAA GTG GCT GAT GAG GTT GAG ACG AGT CCG GTA GCA CAA GCG	192
	49	Asp Pro Glu Val Ala Asp Glu Val Glu Thr Ser Arg Val Ala Gln Ala	64
10	193	ATT GCC AGC ATA CAG CAA TAT ATG ACT CGT CTG GTC AAC GGC TCT GAA	240
	65	Ile Ala Ser Ile Gln Gln Tyr Met Thr Arg Leu Val Asn Gly Ser Glu	80
15	241	CCG GGG CGT CAG GCG ATG GAG CCT TCT ACA GCT AAC GAA TGG CGT GAT	288
	81	Pro Gly Arg Gln Ala Met Glu Pro Ser Thr Ala Asn Glu Trp Arg Asp	96
20	289	AAT GAT AAC CAA TAT GCT ATC TGG GCT GCG GGG GCT GAG GTT CGA AAT	336
	97	Asn Asp Asn Gln Tyr Ala Ile Trp Ala Ala Gly Ala Glu Val Arg Asn	112
25	337	TAC GCT GAA AAC TAT ATT TCA CCC ATC ACC CGG CAG GAA AAA AGC CAT	384
	113	Tyr Ala Glu Asn Tyr Ile Ser Pro Ile Thr Arg Gln Glu Lys Ser His	128
30	385	TAT TTC TCG GAG CTG GAG ACG ACT TTA AAT CAG AAT CGA CTC GAT CCG	432
	129	Tyr Phe Ser Glu Leu Glu Thr Thr Leu Asn Gln Asn Arg Leu Asp Pro	144
35	433	GAT CGT GTG CAG GAT GCT GTT TTG GCG TAT CTC AAT GAG TTT GAG GCA	480
	145	Asp Arg Val Gln Asp Ala Val Leu Ala Tyr Leu Asn Glu Phe Glu Ala	160
40	481	GTG AGT AAT CTA TAT GTG CTC AGT GGT TAT ATT AAT CAG GAT AAA TTT	528
	161	Val Ser Asn Leu Tyr Val Leu Ser Gly Tyr Ile Asn Gln Asp Lys Phe	176
45	529	GAC CAA GCT ATC TAC TAC TTT ATT GGT CGC ACT ACC ACT AAA CCG TAT	576
	177	Asp Gln Ala Ile Tyr Tyr Phe Ile Gly Arg Thr Thr Thr Lys Pro Tyr	192
50	577	CGC TAC TAC TGG CGT CAG ATG GAT TTG AGT AAG AAC CGT CAA GAT CCG	624
	193	Arg Tyr Tyr Trp Arg Gln Met Asp Leu Ser Lys Asn Arg Gln Asp Pro	208
55	625	GCA GGG AAT CCG GTG ACG CCA AAT TGC TGG AAT GAT TGG CAG GAA ATC	672
	209	Ala Gly Asn Pro Val Thr Pro Asn Cys Trp Asn Asp Trp Gln Glu Ile	224
60	673	ACT TTG CCG CTG TCT GGT GAT ACG GTG CTG GAG CAT ACA GTT CGC CCG	720
	225	Thr Leu Pro Leu Ser Gly Asp Thr Val Leu Glu His Thr Val Arg Pro	240
65	721	GTA TTT TAT AAT GAT CGA CTA TAT GTG GCT TGG GTT GAG CGT GAC CCG	768
	241	Val Phe Tyr Asn Asp Arg Leu Tyr Val Ala Trp Val Glu Arg Asp Pro	256
70	769	GCA GTA CAG AAG GAT GCT GAC GGT AAA AAC ATC GGT AAA ACC CAT GCC	816
	257	Ala Val Gln Lys Asp Ala Asp Gly Lys Asn Ile Gly Lys Thr His Ala	272
75	817	TAC AAC ATA AAG TTT GGT TAT AAA CGT TAT GAT GAT ACT TGG ACA GCG	864
	273	Tyr Asn Ile Lys Phe Gly Tyr Lys Arg Tyr Asp Asp Thr Trp Thr Ala	288
80	865	CCG AAT ACG ACC ACG TTA ATG ACA CAA CAA GCA GGG GAA AGT TCA GAA	912
	289	Pro Asn Thr Thr Thr Leu Met Thr Gln Gln Ala Gly Glu Ser Ser Glu	324

5	913	ACA	CAG	CGA	TCC	AGC	CTG	CTG	ATT	GAT	GAA	TCT	AGC	ACC	ACA	TTG	CGC	960
	305	Thr	Gln	Arg	Ser	Ser	Leu	Leu	Ile	Asp	Glu	Ser	Ser	Thr	Thr	Leu	Arg	320
10	961	CAA	GTT	AAT	CTG	TTG	GCT	ACC	ACC	GAT	TTT	AGT	ATC	GAT	CCG	ACG	GAG	1008
	321	Gln	Val	Asn	Leu	Leu	Ala	Thr	Thr	Asp	Phe	Ser	Ile	Asp	Pro	Thr	Glu	336
15	1009	GAA	ACG	GAC	AGT	AAC	CCG	TAT	GGC	CGC	CTA	ATG	TTG	GGG	GTG	TTT	GTC	1056
	337	Glu	Thr	Asp	Ser	Asn	Pro	Tyr	Gly	Arg	Leu	Met	Leu	Gly	Val	Phe	Val	352
20	1057	CGT	CAA	TTT	GAA	GGT	GAT	GGG	GCC	AAT	AGA	AAA	AAT	AAA	CCC	GTT	GTT	1104
	353	Arg	Gln	Phe	Glu	Gly	Asp	Gly	Ala	Asn	Arg	Lys	Asn	Lys	Pro	Val	Val	368
25	1105	TAT	GGT	TAT	CTC	TAT	TGT	GAC	TCA	GCT	TTC	AAT	CGT	CAT	GTT	CTC	AGG	1152
	369	Tyr	Gly	Tyr	Leu	Tyr	Cys	Asp	Ser	Ala	Phe	Asn	Arg	His	Val	Leu	Arg	384
30	1153	CCG	TTA	AGT	AAG	AAC	TTT	TTG	TTC	AGT	ACT	TAC	CGT	GAT	GAA	ACG	GAT	1200
	385	Pro	Leu	Ser	Lys	Asn	Phe	Leu	Phe	Ser	Thr	Tyr	Arg	Asp	Glu	Thr	Asp	400
35	1201	GGT	CAA	AAC	AGC	TTG	CAA	TTT	GCG	GTA	TAC	GAT	AAA	AAG	TAT	GTA	ATT	1248
	401	Gly	Gln	Asn	Ser	Leu	Gln	Phe	Ala	Val	Tyr	Asp	Lys	Lys	Tyr	Val	Ile	416
40	1249	ACT	AAG	GTT	GTT	ACA	GGT	GCA	ACG	GAA	GAT	CCC	GAA	AAT	ACA	GGA	TGG	1296
	417	Thr	Lys	Val	Val	Thr	Gly	Ala	Thr	Glu	Asp	Pro	Glu	Asn	Thr	Gly	Trp	432
45	1297	GTA	AGT	AAA	GTT	GAT	GAC	TTG	AAA	CAA	GGC	ACT	ACT	GGG	GCC	TAT	GTG	1344
	433	Val	Ser	Lys	Val	Asp	Asp	Leu	Lys	Gln	Gly	Thr	Thr	Gly	Ala	Tyr	Val	448
50	1345	TAT	ATC	GAT	CAA	GAT	GGC	CTG	ACG	CTT	CAT	ATA	CAA	ACC	ACA	ACT	AAT	1392
	449	Tyr	Ile	Asp	Gln	Asp	Gly	Leu	Thr	Leu	His	Ile	Gln	Thr	Thr	Thr	Asn	464
55	1393	GGG	GAT	TTT	ATT	AAC	CGT	CAT	ACG	TTT	GGA	TAT	AAC	GAT	CTT	GTA	TAT	1440
	465	Gly	Asp	Phe	Ile	Asn	Arg	His	Thr	Phe	Gly	Tyr	Asn	Asp	Leu	Val	Tyr	480
60	1441	GAT	TCT	AAG	TCT	GGT	TAT	GGT	TTC	ACG	TGG	TCA	GGA	AAT	GAA	GGT	TTT	1488
	481	Asp	Ser	Lys	Ser	Gly	Tyr	Gly	Phe	Thr	Trp	Ser	Gly	Asn	Glu	Gly	Phe	496
65	1489	TAT	CTG	GAT	TAC	CAT	GAT	GGA	AAT	TAT	TAC	ACC	TTT	CAT	AAT	GCA	ATA	1536
	497	Tyr	Leu	Asp	Tyr	His	Asp	Gly	Asn	Tyr	Tyr	Thr	Phe	His	Asn	Ala	Ile	512
70	1537	ATC	AAC	TAC	TAT	CCG	TCT	GGA	TAT	GGT	GGT	GGA	TCT	GTT	CCT	AAT	GGA	1584
	513	Ile	Asn	Tyr	Tyr	Pro	Ser	Gly	Tyr	Gly	Gly	Gly	Ser	Val	Pro	Asn	Gly	523
75	1585	ACG	TGG	GCG	TTA	GAG	CAA	AGG	ATT	AAT	GAG	GGA	TGG	GCT	ATT	GCT	CCC	1632
	529	Thr	Trp	Ala	Leu	Glu	Gln	Arg	Ile	Asn	Glu	Gly	Trp	Ala	Ile	Ala	Pro	544
80	1633	CTG	CTT	GAT	ACT	CTC	CAT	ACT	GTT	ACT	GTG	AAG	GGC	AGT	TAT	ATC	GCT	1680
	545	Leu	Leu	Asp	Thr	Leu	His	Thr	Val	Thr	Val	Lys	Gly	Ser	Tyr	Ile	Ala	560



	1631	TGG	GAA	GGG	GAA	ACA	CCT	ACC	GGT	TAT	AAT	CTG	TAT	ATT	CCA	GAT	GGT	1728
	561	Trp	Glu	Gly	Glu	Thr	Pro	Thr	Gly	Tyr	Asn	Leu	Tyr	Ile	Pro	Asp	Gly	576
5	1729	ACC	GTG	TTG	CTA	GAT	TGG	TTT	GAT	AAA	ATA	AAT	TTT	GCT	ATT	GGT	CTT	1776
	577	Thr	Val	Leu	Leu	Asp	Trp	Phe	Asp	Lys	Ile	Asn	Phe	Ala	Ile	Gly	Leu	592
10	1777	AAT	AAG	CTT	GAG	TCT	GTA	TTT	ACG	TCG	CCA	GAT	TGG	CCA	ACA	CTA	ACC	1824
	593	Asn	Lys	Leu	Glu	Ser	Val	Phe	Thr	Ser	Pro	Asp	Trp	Pro	Thr	Leu	Thr	608
15	1825	ACT	ATC	AAA	AAT	TTC	AGT	AAA	ATC	GCC	GAT	AAC	CGC	AAA	TTC	TAT	CAG	1872
	609	Thr	Ile	Lys	Asn	Phe	Ser	Lys	Ile	Ala	Asp	Asn	Arg	Lys	Phe	Tyr	Gln	624
	1873	GAA	ATC	AAT	GCT	GAG	ACG	GCG	GAT	GGA	CGC	AAC	CTG	TTT	AAA	CGT	TAC	1920
	625	Glu	Ile	Asn	Ala	Glu	Thr	Ala	Asp	Gly	Arg	Asn	Leu	Phe	Lys	Arg	Tyr	640
20	1921	AGT	ACT	CAA	ACT	TTC	GGA	CTT	ACC	AGC	GGT	GCG	ACT	TAT	TCT	ACA	ACT	1968
	641	Ser	Thr	Gln	Thr	Phe	Gly	Leu	Thr	Ser	Gly	Ala	Thr	Tyr	Ser	Thr	Thr	656
25	1969	TAT	ACT	TTG	TCT	GAG	GCG	GAT	TTC	TCC	ACT	GAT	CCG	GAC	AAA	AAC	TAC	2016
	657	Tyr	Thr	Leu	Ser	Glu	Ala	Asp	Phe	Ser	Thr	Asp	Pro	Asp	Lys	Asn	Tyr	672
30	2017	CTA	CAG	GTT	TGT	TTG	AAT	GTC	GTG	TGG	GAT	CAT	TAT	GAC	CGC	CCG	TCA	2064
	673	Leu	Gln	Val	Cys	Leu	Asn	Val	Val	Trp	Asp	His	Tyr	Asp	Arg	Pro	Ser	688
35	2065	GGG	AAA	AAA	GGG	GCT	TAT	TCT	TGG	GTC	AGT	AAG	TGG	TTT	AAC	GTC	TAT	2112
	689	Gly	Lys	Lys	Gly	Ala	Tyr	Ser	Trp	Val	Ser	Lys	Trp	Phe	Asn	Val	Tyr	704
	2113	GTT	GCG	TTG	CAA	GAT	AGC	AAA	GCT	CCG	GAT	GCC	ATT	CCT	CGA	TTA	GTT	2160
	705	Val	Ala	Leu	Gln	Asp	Ser	Lys	Ala	Pro	Asp	Ala	Ile	Pro	Arg	Leu	Val	720
40	2161	TCC	CGT	TAC	GAT	AGT	AAA	CGT	GGT	CTG	GTG	CAA	TAT	CTG	GAC	TTC	TGG	2208
	721	Ser	Arg	Tyr	Asp	Ser	Lys	Arg	Gly	Leu	Val	Gln	Tyr	Leu	Asp	Phe	Trp	736
45	2209	ACC	TCA	TCA	TTA	CCC	GCG	AAA	ACC	CGT	CTT	AAC	ACC	ACC	TTT	GTG	CGT	2256
	737	Thr	Ser	Ser	Leu	Pro	Ala	Lys	Thr	Arg	Leu	Asn	Thr	Thr	Phe	Val	Arg	752
50	2257	ACT	TTG	ATT	GAG	AAG	GCT	AAT	CTG	GGG	CTG	GAT	AGT	TTG	CTG	GAT	TAC	2304
	753	Thr	Leu	Ile	Glu	Lys	Ala	Asn	Leu	Gly	Leu	Asp	Ser	Leu	Leu	Asp	Tyr	768
55	2305	ACC	TTG	CAG	GCA	GAT	CCT	TCT	CTG	GAA	GCA	GAT	TTA	GTG	ACT	GAC	GGC	2352
	769	Thr	Leu	Gln	Ala	Asp	Pro	Ser	Leu	Glu	Ala	Asp	Leu	Val	Thr	Asp	Gly	784
	2353	AAA	AGC	GAA	CCA	ATG	GAC	TTT	AAT	GGT	TCA	AAC	GGT	CTC	TAT	TTC	TGG	2400
	785	Lys	Ser	Glu	Pro	Met	Asp	Phe	Asn	Gly	Ser	Asn	Gly	Leu	Tyr	Phe	Trp	300
60	2401	GAA	TTG	TTC	TTT	CAC	CTG	CCG	TTT	TTG	GTT	GCT	ACA	CGC	TTT	GCC	AAC	2448
	801	Glu	Leu	Phe	Phe	His	Leu	Pro	Phe	Leu	Val	Ala	Thr	Arg	Phe	Ala	Asn	316
65	2449	GAA	CAG	CAA	TTT	TCG	CCG	GCA	CAA	AAG	AGT	TTG	CAT	TAC	ATC	TTT	GAC	2496
	817	Glu	Gln	Gln	Phe	Ser	Pro	Ala	Gln	Lys	Ser	Leu	His	Tyr	Ile	Phe	Asp	332

5	2497	CCG GCG ATG AAA AAC AAG CCA CAC AAT GCC CCG GCT TAT TGG AAT GTA	2544
	333	Pro Ala Met Lys Asn Lys Pro His Asn Ala Pro Ala Tyr Trp Asn Val	348
10	2545	CGT CCG TTG GTT GAA GGA AAC AGC GAT TTG TCA CGT CAT TTG GAC GAT	2592
	349	Arg Pro Leu Val Glu Gly Asn Ser Asp Leu Ser Arg His Leu Asp Asp	364
15	2593	TCT ATA GAC CCA GAT ACT CAA GCT TAT GCT CAT CCG GTG ATA TAC CAG	2640
	365	Ser Ile Asp Pro Asp Thr Gln Ala Tyr Ala His Pro Val Ile Tyr Gln	380
20	2641	AAA GCG GTG TTT ATT GCC TAT GTC AGT AAC CTG ATT GCT CAG GGA GAT	2688
	381	Lys Ala Val Phe Ile Ala Tyr Val Ser Asn Leu Ile Ala Gln Gly Asp	396
25	2689	ATG TGG TAT CGC CAA TTG ACT CGT GAC GGT CTG ACT CAG GCC CGT GTC	2736
	397	Met Trp Tyr Arg Gln Leu Thr Arg Asp Gly Leu Thr Gln Ala Arg Val	412
30	2737	TAT TAC AAT CTG GCC GCT GAA TTG CTA GGG CCT CGT CCG GAT GTA TCG	2784
	413	Tyr Tyr Asn Leu Ala Ala Glu Leu Leu Gly Pro Arg Pro Asp Val Ser	428
35	2785	CTG AGT AGC ATT TGG ACG CCG CAA ACC CTG GAT ACC TTA GCA GCC GGG	2832
	429	Leu Ser Ser Ile Trp Thr Pro Gln Thr Leu Asp Thr Leu Ala Ala Gly	444
40	2833	CAA AAA GCG GTT TTA CGT GAT TTT GAG CAC CAG TTG GCT AAT AGT GAT	2880
	445	Gln Lys Ala Val Leu Arg Asp Phe Glu His Gln Leu Ala Asn Ser Asp	460
45	2881	ACC GCT TTA CCC GCA TTG CCG GGC CGC AAT GTC AGC TAC TTG AAA CTG	2928
	461	Thr Ala Leu Pro Ala Leu Pro Gly Arg Asn Val Ser Tyr Leu Lys Leu	476
50	2929	GCA GAT AAT GGC TAC TTT AAT GAA CCG CTC AAT GTT CTG ATG TTG TCT	2976
	477	Ala Asp Asn Gly Tyr Phe Asn Glu Pro Leu Asn Val Leu Met Leu Ser	492
55	2977	CAC TGG GAT ACG TTG GAT GCA CGG TTA TAC AAT CTG CGT CAT AAC CTG	3024
	493	His Trp Asp Thr Leu Asp Ala Arg Leu Tyr Asn Leu Arg His Asn Leu	1008
60	3025	ACC GTT GAT GGC AAG CCG CTT TCG CTG CCG CTG TAT GCT GCG CCT GTT	3072
	1009	Thr Val Asp Gly Lys Pro Leu Ser Leu Pro Leu Tyr Ala Ala Pro Val	1024
65	3073	GAT CCG GTA GCG TTG TTG GCT CAG CGT GCT CAG TCC GGC ACG TTG ACG	3120
	1025	Asp Pro Val Ala Leu Leu Ala Gln Arg Ala Gln Ser Gly Thr Leu Thr	1040
70	3121	AAT GGC GTC AGT GGC GCC ATG TTG ACG GTG CCG CCA TAC CGT TTC AGC	3168
	1041	Asn Gly Val Ser Gly Ala Met Leu Thr Val Pro Pro Tyr Arg Phe Ser	1056
75	3169	GCT ATG TTG CCG CGA GCT TAC AGC GCC GTG GGT ACG TTG ACC AGT TTT	3216
	1057	Ala Met Leu Pro Arg Ala Tyr Ser Ala Val Gly Thr Leu Thr Ser Phe	1072
80	3217	GGT CAG AAC CTG CTT AGT TTG TTG GAA CGT AGC GAA CGA GCC TGT CAA	3264
	1073	Gly Gln Asn Leu Leu Ser Leu Leu Glu Arg Ser Glu Arg Ala Cys Gln	1088

	3265	GAA	GAG	TTG	GCG	CAA	CAG	CAA	CTG	TTG	GAT	ATG	TCC	AGC	TAT	GCG	ATC	3311
	1089	Glu	Glu	Leu	Ala	Gln	Gln	Gln	Leu	Leu	Asp	Met	Ser	Ser	Tyr	Ala	Ile	1104
5	3313	ACG	TTG	CAA	CAA	CAG	GCG	CTG	GAT	GGA	TTG	GCG	GCA	GAT	CGT	CTG	GCG	3369
	1105	Thr	Leu	Gln	Gln	Gln	Ala	Leu	Asp	Gly	Leu	Ala	Ala	Asp	Arg	Leu	Ala	1120
10	3361	CTG	CTA	GCT	AGT	CAG	GCT	ACG	GCA	CAA	CAG	CGT	CAT	GAC	CAT	TAT	TAC	3403
	1121	Leu	Leu	Ala	Ser	Gln	Ala	Thr	Ala	Gln	Gln	Arg	His	Asp	His	Tyr	Tyr	1156
15	3409	ACT	CTG	TAT	CAG	AAC	AAC	ATC	TCC	AGT	GCG	GAA	CAA	CTG	GTG	ATG	GAC	3456
	1137	Thr	Leu	Tyr	Gln	Asn	Asn	Ile	Ser	Ser	Ala	Glu	Gln	Leu	Val	Met	Asp	1152
20	3457	ACC	CAA	ACG	TCA	GCA	CAA	TCC	CTG	ATT	TCT	TCT	TCC	ACT	GGT	GTA	CAA	3504
	1153	Thr	Gln	Thr	Ser	Ala	Gln	Ser	Leu	Ile	Ser	Ser	Ser	Thr	Gly	Val	Gln	1168
25	3505	ACT	GCC	AGT	GGG	GCA	CTG	AAA	GTG	ATC	CCG	AAT	ATC	TTT	GGT	TTG	GCT	3552
	1169	Thr	Ala	Ser	Gly	Ala	Leu	Lys	Val	Ile	Pro	Asn	Ile	Phe	Gly	Leu	Ala	1184
30	3553	GAT	GGC	GGC	TCG	CGC	TAT	GAA	GGA	GTA	ACG	GAA	GCG	ATT	GCC	ATC	GGG	3600
	1185	Asp	Gly	Gly	Ser	Arg	Tyr	Glu	Gly	Val	Thr	Glu	Ala	Ile	Ala	Ile	Gly	1200
35	3601	TTA	ATG	GCT	GCC	GGA	CAA	GCC	ACC	AGC	GTG	GTG	GCC	GAG	CGT	CTG	GCA	3648
	1201	Leu	Met	Ala	Ala	Gly	Gln	Ala	Thr	Ser	Val	Val	Ala	Glu	Arg	Leu	Ala	1216
40	3649	ACC	ACG	GAG	AAT	TAC	CGC	CGC	CGC	CGT	GAA	GAG	TGG	CAA	ATC	CAA	TAC	3696
	1217	Thr	Thr	Glu	Asn	Tyr	Arg	Arg	Arg	Arg	Glu	Glu	Trp	Gln	Ile	Gln	Tyr	1232
45	3697	CAG	CAG	GCA	CAG	TCT	GAG	GTC	GAC	GCA	TTA	CAG	AAA	CAG	TTG	GAT	GCG	3744
	1233	Gln	Gln	Ala	Gln	Ser	Glu	Val	Asp	Ala	Leu	Gln	Lys	Gln	Leu	Asp	Ala	1248
50	3745	CTG	GCA	GTG	CGC	GAG	AAA	GCA	GCT	CAA	ACT	TCC	CTG	CAA	CAG	GCG	AAG	3792
	1249	Leu	Ala	Val	Arg	Glu	Lys	Ala	Ala	Gln	Thr	Ser	Leu	Gln	Gln	Ala	Lys	1264
55	3793	GCA	CAG	CAG	GTA	CAA	ATT	CGG	ACC	ATG	CTG	ACT	TAC	TTA	ACT	ACT	CGT	3840
	1265	Ala	Gln	Gln	Val	Gln	Ile	Arg	Thr	Met	Leu	Thr	Tyr	Leu	Thr	Thr	Arg	1280
60	3841	TTC	ACC	CAG	GCG	ACT	CTG	TAC	CAG	TGG	CTG	AGT	GGT	CAA	TTA	TCC	GCG	3888
	1281	Phe	Thr	Gln	Ala	Thr	Leu	Tyr	Gln	Trp	Leu	Ser	Gly	Gln	Leu	Ser	Ala	1296
65	3889	TTG	TAT	TAT	CAA	GCG	TAT	GAT	GCC	GTG	GTT	GCT	CTC	TGC	CTC	TCC	GCC	3936
	1297	Leu	Tyr	Tyr	Gln	Ala	Tyr	Asp	Ala	Val	Val	Ala	Leu	Cys	Leu	Ser	Ala	1312
70	3937	CAA	GCT	TGC	TGG	CAG	TAT	GAA	TTG	GGT	GAT	TAC	GCT	ACC	ACT	TTT	ATC	3984
	1313	Gln	Ala	Cys	Trp	Gln	Tyr	Glu	Leu	Gly	Asp	Tyr	Ala	Thr	Thr	Phe	Ile	1328
75	3985	CAG	ACC	GGT	ACC	TGG	AAC	GAC	CAT	TAC	CGT	GGT	TTG	CAA	GTG	GGG	GAG	4032
	1329	Gln	Thr	Gly	Thr	Trp	Asn	Asp	His	Tyr	Arg	Gly	Leu	Gln	Val	Gly	Glu	1344
80	4033	ACA	CTG	CAA	CTC	AAT	TTG	CAT	CAG	ATG	GAA	GCG	GCC	TAT	TTA	GTT	CGT	4080
	1345	Thr	Leu	Gln	Leu	Asn	Leu	His	Gln	Met	Glu	Ala	Ala	Tyr	Leu	Val	Arg	1360

5  
 4081 CAC GAA CGC CGT CTT AAT GTG ATC GGT ACT GTG TCG CTC AAA AGC CTA 4123  
 1361 His Glu Arg Arg Leu Asn Val Ile Arg Thr Val Ser Leu Lys Ser Leu 1376

10  
 4129 TTG GGT GAT GAT GGT TTT GGT AAG TTA AAA ACC GAA GGC AAA GTC GAC 4176  
 1377 Leu Gly Asp Asp Gly Phe Gly Lys Leu Lys Thr Glu Gly Lys Val Asp 1392

15  
 4177 TTT CCA TTA AGC GAA AAG CTG TTT GAC AAC GAC TAT CCG GGG CAC TAT 4224  
 1393 Phe Pro Leu Ser Glu Lys Leu Phe Asp Asn Asp Tyr Pro Gly His Tyr 1408

20  
 4225 TTG CGC CAG ATT AAA ACT GTG TCA GTG ACG TTG CCG ACG TTA GTC GGG 4272  
 1409 Leu Arg Gln Ile Lys Thr Val Ser Val Thr Leu Pro Thr Leu Val Gly 1424

25  
 4273 CCG TAT CAA AAC GTG AAG GCA ACG CTC ACT CAG ACC AGC AGC AGT ATA 4320  
 1425 Pro Tyr Gln Asn Val Lys Ala Thr Leu Thr Gln Thr Ser Ser Ser Ile 1440

30  
 4321 TTG TTA GCA GCA GAT ATC AAT GGT GTT AAA CGT CTC AAT GAT CCG ACA 4368  
 1441 Leu Leu Ala Ala Asp Ile Asn Gly Val Lys Arg Leu Asn Asp Pro Thr 1456

35  
 4369 GGT AAA GAG GGT GAT GCG ACG CAT ATT GTC ACC AAT CTG CGT GCC AGC 4416  
 1457 Gly Lys Glu Gly Asp Ala Thr His Ile Val Thr Asn Leu Arg Ala Ser 1472

40  
 4417 CAG CAG GTG GCG CTC TCT TCT GGC ATT AAT GAT GCC GGT AGC TTT GAG 4464  
 1473 Gln Gln Val Ala Leu Ser Ser Gly Ile Asn Asp Ala Gly Ser Phe Glu 1488

45  
 4465 TTG CGT TTG GAA GAT GAG CGC TAT CTA TCA TTT GAG GGG ACT GGA GCT 4512  
 1489 Leu Arg Leu Glu Asp Glu Arg Tyr Leu Ser Phe Glu Gly Thr Gly Ala 1504

50  
 4513 GTT TCC AAA TGG ACT CTT AAC TTC CCG CGT TCT GTG GAT GAG CAT ATT 4560  
 1505 Val Ser Lys Trp Thr Leu Asn Phe Pro Arg Ser Val Asp Glu His Ile 1520

55  
 4561 GAC GAT AAG ACA TTG AAA GCG GAT GAG ATG CAG GCC GCA CTG TTG GCG 4608  
 1521 Asp Asp Lys Thr Leu Lys Ala Asp Glu Met Gln Ala Ala Leu Leu Ala 1536

60  
 4609 AAT ATG GAT GAT GTG CTG GTG CAG GTG CAT TAT ACC GCC TGC GAC GGC 4656  
 1537 Asn Met Asp Asp Val Leu Val Gln Val His Tyr Thr Ala Cys Asp Gly 1552

65  
 4657 GGC GCC AGT TTC GCA AAC CAG GTC AAG AAA ACA CTC TCT TAA 4698  
 1553 Gly Ala Ser Phe Ala Asn Gln Val Lys Lys Thr Leu Ser End 1566

55 (2) INFORMATION FOR SEQ ID NO:59  
 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1665 amino acids  
 (B) TYPE: amino acid  
 (C) TOPOLOGY: linear

60 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59 (TccB peptide)

65 Features From To Description

1 11 SEQ ID NO:7

5	1	Met Leu Ser Thr Met Glu Lys Gln Leu Asn Glu Ser Gln Arg Asp Ala	16
	17	Leu Val Thr Gly Tyr Met Asn Phe Val Ala Pro Thr Leu Lys Gly Val	32
	33	Ser Gly Gln Pro Val Thr Val Glu Asp Leu Tyr Glu Tyr Leu Leu Ile	48
10	49	Asp Pro Glu Val Ala Asp Glu Val Glu Thr Ser Arg Val Ala Gln Ala	64
	65	Ile Ala Ser Ile Gln Gln Tyr Met Thr Arg Leu Val Asn Gly Ser Glu	80
15	81	Pro Gly Arg Gln Ala Met Glu Pro Ser Thr Ala Asn Glu Trp Arg Asp	96
	97	Asn Asp Asn Gln Tyr Ala Ile Trp Ala Ala Gly Ala Glu Val Arg Asn	112
	113	Tyr Ala Glu Asn Tyr Ile Ser Pro Ile Thr Arg Gln Glu Lys Ser His	128
20	129	Tyr Phe Ser Glu Leu Glu Thr Thr Leu Asn Gln Asn Arg Leu Asp Pro	144
	145	Asp Arg Val Gln Asp Ala Val Leu Ala Tyr Leu Asn Glu Phe Glu Ala	160
25	161	Val Ser Asn Leu Tyr Val Leu Ser Gly Tyr Ile Asn Gln Asp Lys Phe	176
	177	Asp Gln Ala Ile Tyr Tyr Phe Ile Gly Arg Thr Thr Thr Lys Pro Tyr	192
	193	Arg Tyr Tyr Trp Arg Gln Met Asp Leu Ser Lys Asn Arg Gln Asp Pro	208
30	209	Ala Gly Asn Pro Val Thr Pro Asn Cys Trp Asn Asp Trp Gln Glu Ile	224
	225	Thr Leu Pro Leu Ser Gly Asp Thr Val Leu Glu His Thr Val Arg Pro	240
35	241	Val Phe Tyr Asn Asp Arg Leu Tyr Val Ala Trp Val Glu Arg Asp Pro	256
	257	Ala Val Gln Lys Asp Ala Asp Gly Lys Asn Ile Gly Lys Thr His Ala	272
	273	Tyr Asn Ile Lys Phe Gly Tyr Lys Arg Tyr Asp Asp Thr Trp Thr Ala	288
40	289	Pro Asn Thr Thr Thr Leu Met Thr Gln Gln Ala Gly Glu Ser Ser Glu	304
	305	Thr Gln Arg Ser Ser Leu Leu Ile Asp Glu Ser Ser Thr Thr Leu Arg	320
45	321	Gln Val Asn Leu Leu Ala Thr Thr Asp Phe Ser Ile Asp Pro Thr Glu	336
	337	Glu Thr Asp Ser Asn Pro Tyr Gly Arg Leu Met Leu Gly Val Phe Val	352
	353	Arg Gln Phe Glu Gly Asp Gly Ala Asn Arg Lys Asn Lys Pro Val Val	368
50	369	Tyr Gly Tyr Leu Tyr Cys Asp Ser Ala Phe Asn Arg His Val Leu Arg	384
	385	Pro Leu Ser Lys Asn Phe Leu Phe Ser Thr Tyr Arg Asp Glu Thr Asp	400
55	401	Gly Gln Asn Ser Leu Gln Phe Ala Val Tyr Asp Lys Lys Tyr Val Ile	416
	417	Thr Lys Val Val Thr Gly Ala Thr Glu Asp Pro Glu Asn Thr Gly Trp	432
	433	Val Ser Lys Val Asp Asp Leu Lys Gln Gly Thr Thr Gly Ala Tyr Val	448
60	449	Tyr Ile Asp Gln Asp Gly Leu Thr Leu His Ile Gln Thr Thr Thr Asn	464
	465	Gly Asp Phe Ile Asn Arg His Thr Phe Gly Tyr Asn Asp Leu Val Tyr	480
65	481	Asp Ser Lys Ser Gly Tyr Gly Phe Thr Trp Ser Gly Asn Glu Gly Phe	496
	497	Tyr Leu Asp Tyr His Asp Gly Asn Tyr Tyr Thr Phe His Asn Ala Ile	512

	513	Ile Asn Tyr Tyr Pro Ser Gly Tyr Gly Gly Gly Ser Val Pro Asn Gly	523
5	529	Thr Trp Ala Leu Glu Gln Arg Ile Asn Glu Gly Trp Ala Ile Ala Pro	544
	545	Leu Leu Asp Thr Leu His Thr Val Thr Val Lys Gly Ser Tyr Ile Ala	560
	561	Trp Glu Gly Glu Thr Pro Thr Gly Tyr Asn Leu Tyr Ile Pro Asp Gly	576
10	577	Thr Val Leu Leu Asp Trp Phe Asp Lys Ile Asn Phe Ala Ile Gly Leu	592
	593	Asn Lys Leu Glu Ser Val Phe Thr Ser Pro Asp Trp Pro Thr Leu Thr	608
15	609	Thr Ile Lys Asn Phe Ser Lys Ile Ala Asp Asn Arg Lys Phe Tyr Gln	624
	625	Glu Ile Asn Ala Glu Thr Ala Asp Gly Arg Asn Leu Phe Lys Arg Tyr	640
	641	Ser Thr Gln Thr Phe Gly Leu Thr Ser Gly Ala Thr Tyr Ser Thr Thr	656
20	657	Tyr Thr Leu Ser Glu Ala Asp Phe Ser Thr Asp Pro Asp Lys Asn Tyr	672
	673	Leu Gln Val Cys Leu Asn Val Val Trp Asp His Tyr Asp Arg Pro Ser	688
25	689	Gly Lys Lys Gly Ala Tyr Ser Trp Val Ser Lys Trp Phe Asn Val Tyr	704
	705	Val Ala Leu Gln Asp Ser Lys Ala Pro Asp Ala Ile Pro Arg Leu Val	720
	721	Ser Arg Tyr Asp Ser Lys Arg Gly Leu Val Gln Tyr Leu Asp Phe Trp	736
30	737	Thr Ser Ser Leu Pro Ala Lys Thr Arg Leu Asn Thr Thr Phe Val Arg	752
	753	Thr Leu Ile Glu Lys Ala Asn Leu Gly Leu Asp Ser Leu Leu Asp Tyr	768
35	769	Thr Leu Gln Ala Asp Pro Ser Leu Glu Ala Asp Leu Val Thr Asp Gly	784
	785	Lys Ser Glu Pro Met Asp Phe Asn Gly Ser Asn Gly Leu Tyr Phe Trp	800
	801	Glu Leu Phe Phe His Leu Pro Phe Leu Val Ala Thr Arg Phe Ala Asn	816
40	817	Glu Gln Gln Phe Ser Pro Ala Gln Lys Ser Leu His Tyr Ile Phe Asp	832
	833	Pro Ala Met Lys Asn Lys Pro His Asn Ala Pro Ala Tyr Trp Asn Val	848
45	849	Arg Pro Leu Val Glu Gly Asn Ser Asp Leu Ser Arg His Leu Asp Asp	864
	865	Ser Ile Asp Pro Asp Thr Gln Ala Tyr Ala His Pro Val Ile Tyr Gln	880
	881	Lys Ala Val Phe Ile Ala Tyr Val Ser Asn Leu Ile Ala Gln Gly Asp	896
50	897	Met Trp Tyr Arg Gln Leu Thr Arg Asp Gly Leu Thr Gln Ala Arg Val	912
	913	Tyr Tyr Asn Leu Ala Ala Glu Leu Leu Gly Pro Arg Pro Asp Val Ser	928
55	929	Leu Ser Ser Ile Trp Thr Pro Gln Thr Leu Asp Thr Leu Ala Ala Gly	944
	945	Gln Lys Ala Val Leu Arg Asp Phe Glu His Gln Leu Ala Asn Ser Asp	960
	961	Thr Ala Leu Pro Ala Leu Pro Gly Arg Asn Val Ser Tyr Leu Lys Leu	976
60	977	Ala Asp Asn Gly Tyr Phe Asn Glu Pro Leu Asn Val Leu Met Leu Ser	992
	993	His Trp Asp Thr Leu Asp Ala Arg Leu Tyr Asn Leu Arg His Asn Leu	1008
65	1009	Thr Val Asp Gly Lys Pro Leu Ser Leu Pro Leu Tyr Ala Ala Pro Val	1024
	1025	Asp Pro Val Ala Leu Leu Ala Gln Arg Ala Gln Ser Gly Thr Leu Thr	1040

	1041	Asn Gly Val Ser Gly Ala Met Leu Thr Val Pro Pro Tyr Arg Phe Ser	1056
5	1057	Ala Met Leu Pro Arg Ala Tyr Ser Ala Val Gly Thr Leu Thr Ser Phe	1070
	1073	Gly Gln Asn Leu Leu Ser Leu Leu Glu Arg Ser Glu Arg Ala Cys Gln	1088
	1089	Glu Glu Leu Ala Gln Gln Gln Leu Leu Asp Met Ser Ser Tyr Ala Ile	1104
10	1105	Thr Leu Gln Gln Gln Ala Leu Asp Gly Leu Ala Ala Asp Arg Leu Ala	1120
	1121	Leu Leu Ala Ser Gln Ala Thr Ala Gln Gln Arg His Asp His Tyr Tyr	1136
15	1137	Thr Leu Tyr Gln Asn Asn Ile Ser Ser Ala Glu Gln Leu Val Met Asp	1152
	1153	Thr Gln Thr Ser Ala Gln Ser Leu Ile Ser Ser Ser Thr Gly Val Gln	1168
	1169	Thr Ala Ser Gly Ala Leu Lys Val Ile Pro Asn Ile Phe Gly Leu Ala	1184
20	1185	Asp Gly Gly Ser Arg Tyr Glu Gly Val Thr Glu Ala Ile Ala Ile Gly	1200
	1201	Leu Met Ala Ala Gly Gln Ala Thr Ser Val Val Ala Glu Arg Leu Ala	1216
25	1217	Thr Thr Glu Asn Tyr Arg Arg Arg Arg Glu Glu Trp Gln Ile Gln Tyr	1232
	1233	Gln Gln Ala Gln Ser Glu Val Asp Ala Leu Gln Lys Gln Leu Asp Ala	1248
	1249	Leu Ala Val Arg Glu Lys Ala Ala Gln Thr Ser Leu Gln Gln Ala Lys	1264
30	1265	Ala Gln Gln Val Gln Ile Arg Thr Met Leu Thr Tyr Leu Thr Thr Arg	1280
	1281	Phe Thr Gln Ala Thr Leu Tyr Gln Trp Leu Ser Gly Gln Leu Ser Ala	1296
35	1297	Leu Tyr Tyr Gln Ala Tyr Asp Ala Val Val Ala Leu Cys Leu Ser Ala	1312
	1313	Gln Ala Cys Trp Gln Tyr Glu Leu Gly Asp Tyr Ala Thr Thr Phe Ile	1328
	1329	Gln Thr Gly Thr Trp Asn Asp His Tyr Arg Gly Leu Gln Val Gly Glu	1344
40	1345	Thr Leu Gln Leu Asn Leu His Gln Met Glu Ala Ala Tyr Leu Val Arg	1360
	1361	His Glu Arg Arg Leu Asn Val Ile Arg Thr Val Ser Leu Lys Ser Leu	1376
45	1377	Leu Gly Asp Asp Gly Phe Gly Lys Leu Lys Thr Glu Gly Lys Val Asp	1392
	1393	Phe Pro Leu Ser Glu Lys Leu Phe Asp Asn Asp Tyr Pro Gly His Tyr	1408
	1409	Leu Arg Gln Ile Lys Thr Val Ser Val Thr Leu Pro Thr Leu Val Gly	1424
50	1425	Pro Tyr Gln Asn Val Lys Ala Thr Leu Thr Gln Thr Ser Ser Ser Ile	1440
	1441	Leu Leu Ala Ala Asp Ile Asn Gly Val Lys Arg Leu Asn Asp Pro Thr	1456
55	1457	Gly Lys Glu Gly Asp Ala Thr His Ile Val Thr Asn Leu Arg Ala Ser	1472
	1473	Gln Gln Val Ala Leu Ser Ser Gly Ile Asn Asp Ala Gly Ser Phe Glu	1488
	1489	Leu Arg Leu Glu Asp Glu Arg Tyr Leu Ser Phe Glu Gly Thr Gly Ala	1504
60	1505	Val Ser Lys Trp Thr Leu Asn Phe Pro Arg Ser Val Asp Glu His Ile	1520
	1521	Asp Asp Lys Thr Leu Lys Ala Asp Glu Met Gln Ala Ala Leu Leu Ala	1536
65	1537	Asn Met Asp Asp Val Leu Val Gln Val His Tyr Thr Ala Cys Asp Gly	1552
	1553	Gly Ala Ser Phe Ala Asn Gln Val Lys Lys Thr Leu Ser	1565

## (2) INFORMATION FOR SEQ ID NO:60

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 3132 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:60 (cacc)

15 1 ATG AGT CCG TCT GAG ACT ACT CTT TAT ACT CAA ACC CCA ACA GTC AGC 48  
 1 Met Ser Pro Ser Glu Thr Thr Leu Tyr Thr Gln Thr Pro Thr Val Ser 16

20 49 GTG TTA GAT AAT CGC GGT CTG TCC ATT CGT GAT ATT GGT TTT CAC CGT 96  
 17 Val Leu Asp Asn Arg Gly Leu Ser Ile Arg Asp Ile Gly Phe His Arg 32

25 97 ATT GTA ATC GGG GGG GAT ACT GAC ACC CGC GTC ACC CGT CAC CAG TAT 144  
 33 Ile Val Ile Gly Gly Asp Thr Asp Thr Arg Val Thr Arg His Gln Tyr 48

30 145 GAT GCC CGT GGA CAC CTG AAC TAC AGT ATT GAC CCA CGC TTG TAT GAT 192  
 49 Asp Ala Arg Gly His Leu Asn Tyr Ser Ile Asp Pro Arg Leu Tyr Asp 64

35 193 GCA AAG CAG GCT GAT AAC TCA GTA AAG CCT AAT TTT GTC TGG CAG CAT 240  
 65 Ala Lys Gln Ala Asp Asn Ser Val Lys Pro Asn Phe Val Trp Gln His 80

40 241 GAT CTG GCC GGT CAT GCC CTG CGG ACA GAG AGT GTC GAT GCT GGT CGT 288  
 81 Asp Leu Ala Gly His Ala Leu Arg Thr Glu Ser Val Asp Ala Gly Arg 96

45 289 ACT GTT GCA TTG AAT GAT ATT GAA GGT CGT TCG GTA ATG ACA ATG AAT 336  
 97 Thr Val Ala Leu Asn Asp Ile Glu Gly Arg Ser Val Met Thr Met Asn 112

50 337 GCG ACC GGT GTT CGT CAG ACC CGT CGC TAT GAA GGC AAC ACC TTG CCC 384  
 113 Ala Thr Gly Val Arg Gln Thr Arg Arg Tyr Glu Gly Asn Thr Leu Pro 128

55 385 GGT CGC TTG TTA TCT GTG AGC GAG CAA GTT TTC AAC CAA GAG AGT GCT 432  
 129 Gly Arg Leu Leu Ser Val Ser Glu Gln Val Phe Asn Gln Glu Ser Ala 144

60 433 AAA GTG ACA GAG CGC TTT ATC TGG GCT GGG AAT ACA ACC TCG GAG AAA 480  
 145 Lys Val Thr Glu Arg Phe Ile Trp Ala Gly Asn Thr Thr Ser Glu Lys 160

65 481 GAG TAT AAC CTC TCC GGT CTG TGT ATA CGC CAC TAC GAC ACA GCG GGA 528  
 161 Glu Tyr Asn Leu Ser Gly Leu Cys Ile Arg His Tyr Asp Thr Ala Gly 176

529 GTG ACC CGG TTG ATG AGT CAG TCA CTG GCG GGC GCC ATG CTA TCC CAA 576  
 177 Val Thr Arg Leu Met Ser Gln Ser Leu Ala Gly Ala Met Leu Ser Gln 192

577 TCT CAC CAA TTG CTG GCG GAA GGG CAG GAG GCT AAC TGG AGC GGT GAC 624  
 193 Ser His Gln Leu Leu Ala Glu Gly Gln Glu Ala Asn Trp Ser Gly Asp 208



	625	GAC GAA ACT GTC TGG CAG GGA ATG CTG GCA AGT GAG GTC TAT ACG ACA	672
	209	Asp Glu Thr Val Trp Gln Gly Met Leu Ala Ser Glu Val Tyr Thr Thr	224
5			
	673	CAA AGT ACC ACT AAT GCC ATC GGG GCT TTA CTG ACC CAA ACC GAT GCG	720
	225	Gln Ser Thr Thr Asn Ala Ile Gly Ala Leu Leu Thr Gln Thr Asp Ala	240
10			
	721	AAA GGC AAT ATT CAG CGT CTG GCT TAT GAC ATT GCC GGT CAG TTA AAA	768
	241	Lys Gly Asn Ile Gln Arg Leu Ala Tyr Asp Ile Ala Gly Gln Leu Lys	256
15			
	769	GGG AGT TGG TTG ACG GTG AAA GGC CAG AGT GAA CAG GTG ATT GTT AAG	816
	257	Gly Ser Trp Leu Thr Val Lys Gly Gln Ser Glu Gln Val Ile Val Lys	272
20			
	817	TCC CTG AGC TGG TCA GCC GCA GGT CAT AAA TTG CGT GAA GAG CAC GGT	864
	273	Ser Leu Ser Trp Ser Ala Ala Gly His Lys Leu Arg Glu Glu His Gly	288
25			
	865	AAC GGC GTG GTT ACG GAG TAC AGT TAT GAG CCG GAA ACT CAA CGT CTG	912
	289	Asn Gly Val Val Thr Glu Tyr Ser Tyr Glu Pro Glu Thr Gln Arg Leu	304
30			
	913	ATA GGT ATC ACC ACC CGG CGT GCC GAA GGG AGT CAA TCA GGA GCC AGA	960
	305	Ile Gly Ile Thr Thr Arg Arg Ala Glu Gly Ser Gln Ser Gly Ala Arg	320
35			
	961	GTA TTG CAG GAT CTA CGC TAT AAG TAT GAT CCG GTG GGG AAT GTT ATC	1008
	321	Val Leu Gln Asp Leu Arg Tyr Lys Tyr Asp Pro Val Gly Asn Val Ile	336
40			
	1009	AGT ATC CAT AAT GAT GCC GAA GCT ACC CGC TTT TGG CGT AAT CAG AAA	1056
	337	Ser Ile His Asn Asp Ala Glu Ala Thr Arg Phe Trp Arg Asn Gln Lys	352
45			
	1057	GTG GAG CCG GAG AAT CGC TAT GTT TAT GAT TCT CTG TAT CAG CTT ATG	1104
	353	Val Glu Pro Glu Asn Arg Tyr Val Tyr Asp Ser Leu Tyr Gln Leu Met	368
50			
	1105	AGT GCG ACA GGG CGT GAA ATG GCT AAT ATC GGT CAG CAA AGC AAC CAA	1152
	369	Ser Ala Thr Gly Arg Glu Met Ala Asn Ile Gly Gln Gln Ser Asn Gln	384
55			
	1153	CTT CCC TCA CCC GTT ATA CCT GTT CCT ACT GAC GAC AGC ACT TAT ACC	1200
	385	Leu Pro Ser Pro Val Ile Pro Val Pro Thr Asp Asp Ser Thr Tyr Thr	400
60			
	1201	AAT TAC CTT CGT ACC TAT ACT TAT GAC CGT GGC GGT AAT TTG GTT CAA	1248
	401	Asn Tyr Leu Arg Thr Tyr Thr Tyr Asp Arg Gly Gly Asn Leu Val Gln	416
65			
	1249	ATC CGA CAC AGT TCA CCC GCG ACT CAA AAT AGT TAC ACC ACA GAT ATC	1296
	417	Ile Arg His Ser Ser Pro Ala Thr Gln Asn Ser Tyr Thr Thr Asp Ile	432
	1297	ACC GTT TCA AGC CGC AGT AAC CGG GCG GTA TTG AGT ACA TTA ACG ACA	1344
	433	Thr Val Ser Ser Arg Ser Asn Arg Ala Val Leu Ser Thr Leu Thr Thr	448
	1345	GAT CCA ACC CGA GTG GAT GCG CTA TTT GAT TCC GGC GGT CAT CAG AAG	1392
	449	Asp Pro Thr Arg Val Asp Ala Leu Phe Asp Ser Gly Gly His Gln Lys	464
	1393	ATG TTA ATA CCG GGG CAA AAT CTG GAT TGG AAT ATT CCG GGT GAA TTC	1440

	455	Met	Leu	Ile	Pro	Gly	Gln	Asn	Leu	Asp	Trp	Asn	Ile	Arg	Gly	Glu	Leu	463
5	1441	CAA	CGA	GTC	ACA	CCG	GTG	AGC	CGT	GAA	AAT	AGC	AGT	GAC	AGT	GAA	TGG	1488
	431	Gln	Arg	Val	Thr	Pro	Val	Ser	Arg	Glu	Asn	Ser	Ser	Asp	Ser	Glu	Trp	496
10	1489	TAT	CGC	TAT	AGC	AGT	GAT	GGC	ATG	CGG	CTG	CTA	AAA	GTG	AGT	GAA	CAG	1536
	497	Tyr	Arg	Tyr	Ser	Ser	Asp	Gly	Met	Arg	Leu	Leu	Lys	Val	Ser	Glu	Gln	512
15	1537	CAG	ACG	GGC	AAC	AGT	ACT	CAA	GTA	CAA	CGG	GTG	ACT	TAT	CTG	CCG	GGA	1584
	513	Gln	Thr	Gly	Asn	Ser	Thr	Gln	Val	Gln	Arg	Val	Thr	Tyr	Leu	Pro	Gly	528
20	1585	TTA	GAG	CTA	CGG	ACA	ACT	GGG	GTT	GCA	GAT	AAA	ACA	ACC	GAA	GAT	TTG	1632
	529	Leu	Glu	Leu	Arg	Thr	Thr	Gly	Val	Ala	Asp	Lys	Thr	Thr	Glu	Asp	Leu	544
25	1633	CAG	GTG	ATT	ACG	GTA	GGT	GAA	GCG	GGT	CGC	GCA	CAG	GTA	AGG	GTA	TTG	1680
	545	Gln	Val	Ile	Thr	Val	Gly	Glu	Ala	Gly	Arg	Ala	Gln	Val	Arg	Val	Leu	560
30	1681	CAC	TGG	GAA	AGT	GGT	AAG	CCG	ACA	GAT	ATT	GAC	AAC	AAT	CAG	GTG	CGC	1728
	561	His	Trp	Glu	Ser	Gly	Lys	Pro	Thr	Asp	Ile	Asp	Asn	Asn	Gln	Val	Arg	576
35	1729	TAC	AGC	TAC	GAT	AAT	CTG	CTT	GGC	TCC	AGC	CAG	CTT	GAA	CTG	GAT	AGC	1776
	577	Tyr	Ser	Tyr	Asp	Asn	Leu	Leu	Gly	Ser	Ser	Gln	Leu	Glu	Leu	Asp	Ser	592
40	1777	GAA	GGG	CAG	ATT	CTC	AGT	CAG	GAA	GAG	TAT	TAT	CCG	TAT	GGC	GGT	ACG	1824
	593	Glu	Gly	Gln	Ile	Leu	Ser	Gln	Glu	Glu	Tyr	Tyr	Pro	Tyr	Gly	Gly	Thr	608
45	1825	GCG	ATA	TGG	GCG	GCG	AGA	AAT	CAG	ACA	GAA	GCC	AGC	TAC	AAA	TTT	ATT	1872
	609	Ala	Ile	Trp	Ala	Ala	Arg	Asn	Gln	Thr	Glu	Ala	Ser	Tyr	Lys	Phe	Ile	624
50	1873	CGT	TAC	TCC	GGT	AAA	GAG	CGG	GAT	GCC	ACT	GGA	TTG	TAT	TAT	TAC	GGC	1920
	625	Arg	Tyr	Ser	Gly	Lys	Glu	Arg	Asp	Ala	Thr	Gly	Leu	Tyr	Tyr	Tyr	Gly	640
55	1921	TAC	CGT	TAT	TAT	CAA	CCT	TGG	GTG	GGT	CGA	TGG	TTG	AGT	GCT	GAT	CCG	1968
	641	Tyr	Arg	Tyr	Tyr	Gln	Pro	Trp	Val	Gly	Arg	Trp	Leu	Ser	Ala	Asp	Pro	656
60	1969	GCG	GGA	ACC	GTG	GAT	GGG	CTG	AAT	TTG	TAC	CGA	ATG	GTG	AGG	AAT	AAC	2016
	657	Ala	Gly	Thr	Val	Asp	Gly	Leu	Asn	Leu	Tyr	Arg	Met	Val	Arg	Asn	Asn	672
65	2017	CCC	ATC	ACA	TTG	ACT	GAC	CAT	GAC	GGA	TTA	GCA	CCG	TCT	CCA	AAT	AGA	2064
	673	Pro	Ile	Thr	Leu	Thr	Asp	His	Asp	Gly	Leu	Ala	Pro	Ser	Pro	Asn	Arg	688
70	2065	AAT	CGA	AAT	ACA	TTT	TGG	TTT	GCT	TCA	TTT	TTG	TTT	CGT	AAA	CCT	GAT	2112
	689	Asn	Arg	Asn	Thr	Phe	Trp	Phe	Ala	Ser	Phe	Leu	Phe	Arg	Lys	Pro	Asp	704
75	2113	GAG	GGA	ATG	TCC	GCG	TCA	ATG	AGA	CGG	GGA	CAA	AAA	ATT	GGC	AGA	GCC	2160
	705	Glu	Gly	Met	Ser	Ala	Ser	Met	Arg	Arg	Gly	Gln	Lys	Ile	Gly	Arg	Ala	720
80	2161	ATT	GCC	GGC	GGG	ATT	GCG	ATT	GCG	GGT	CTT	GCG	GCT	ACC	ATT	GCC	GCT	2208
	721	Ile	Ala	Gly	Gly	Ile	Ala	Ile	Gly	Gly	Leu	Ala	Ala	Thr	Ile	Ala	Ala	736

	2209	ACG GCT GGC GCG GCT ATC CCC GTC ATT CTG GGG GTT GCG GCC GTA GGC	2256
	737	Thr Ala Gly Ala Ala Ile Pro Val Ile Leu Gly Val Ala Ala Val Gly	752
5			
	2257	GCG GGG ATT GGC GCG TTG ATG GGA TAT AAC GTC GGT AGC CTG CTG GAA	2304
	753	Ala Gly Ile Gly Ala Leu Met Gly Tyr Asn Val Gly Ser Leu Leu Glu	768
10			
	2305	AAA GGC GGG GCA TTA CTT GCT CGA CTC GTA CAG GGG AAA TCG ACG TTA	2352
	769	Lys Gly Gly Ala Leu Leu Ala Arg Leu Val Gln Gly Lys Ser Thr Leu	784
15			
	2353	GTA CAG TCG GCG GCT GGC GCG GCT GCC GGA GCG AGT TCA GCC GCG GCT	2400
	785	Val Gln Ser Ala Ala Gly Ala Ala Ala Gly Ala Ser Ser Ala Ala Ala	800
20			
	2401	TAT GGC GCA CGG GCA CAA GGT GTC GGT GTT GCA TCA GCC GCC GGG GCG	2448
	801	Tyr Gly Ala Arg Ala Gln Gly Val Gly Val Ala Ser Ala Ala Gly Ala	816
25			
	2449	GTA ACA GGG GCT GTG GGA TCA TGG ATA AAT AAT GCT GAT CGG GGG ATT	2496
	817	Val Thr Gly Ala Val Gly Ser Trp Ile Asn Asn Ala Asp Arg Gly Ile	832
30			
	2497	GCG GCG GCT ATT GGG GCC GGG AGT GCG GTA GGC ACC ATT GAT ACT ATG	2544
	833	Gly Gly Ala Ile Gly Ala Gly Ser Ala Val Gly Thr Ile Asp Thr Met	848
35			
	2545	TTA GGG ACT GCC TCT ACC CTT ACC CAT GAA GTC GGG GCA GCG GCG GGT	2592
	849	Leu Gly Thr Ala Ser Thr Leu Thr His Glu Val Gly Ala Ala Ala Gly	864
40			
	2593	GGG GCG GCG GGT GGG ATG ATC ACC GGT ACG CAA GGG AGT ACT CGG GCA	2640
	865	Gly Ala Ala Gly Gly Met Ile Thr Gly Thr Gln Gly Ser Thr Arg Ala	880
45			
	2641	GGT ATC CAT GCC GGT ATT GGC ACC TAT TAT GGC TCC TGG ATT GGT TTT	2688
	881	Gly Ile His Ala Gly Ile Gly Thr Tyr Tyr Gly Ser Trp Ile Gly Phe	896
50			
	2689	GGT TTA GAT GTC GCT AGT AAC CCC GCC GGA CAT TTA GCG AAT TAC GCA	2736
	897	Gly Leu Asp Val Ala Ser Asn Pro Ala Gly His Leu Ala Asn Tyr Ala	912
55			
	2737	GTG GGT TAT GCC GCT GGT TTG GGT GCT GAA ATG GCT GTC AAC AGA ATA	2784
	913	Val Gly Tyr Ala Ala Gly Leu Gly Ala Glu Met Ala Val Asn Arg Ile	928
60			
	2785	ATG GGT GGT GGA TTT TTG AGT AGG CTC TTA GGC CGG GTT GTC AGC CCA	2832
	929	Met Gly Gly Gly Phe Leu Ser Arg Leu Leu Gly Arg Val Val Ser Pro	944
65			
	2833	TAT GCC GCC GGT TTA GCC AGA CAA TTA GTA CAT TTC AGT GTC GCC AGA	2880
	945	Tyr Ala Ala Gly Leu Ala Arg Gln Leu Val His Phe Ser Val Ala Arg	960
70			
	2881	CCT GTC TTT GAG CCG ATA TTT AGT GTT CTC GGC GGG CTT GTC GGT GGT	2928
	961	Pro Val Phe Glu Pro Ile Phe Ser Val Leu Gly Gly Leu Val Gly Gly	976
75			
	2929	ATT GGA ACT GGC CTG CAC AGA GTG ATG GGA AGA GAG AGT TGG ATT TCC	2976
	977	Ile Gly Thr Gly Leu His Arg Val Met Gly Arg Glu Ser Trp Ile Ser	992
80			
	2977	AGA GCG TTA AGT GCT GCC GGT AGT GGT ATA GAT CAT GTC GCT GGC ATG	3024

993 Arg Ala Leu Ser Ala Ala Gly Ser Gly Ile Asp His Val Ala Gly Met 1003  
 3025 ATT GGT AAT CAG ATC AGA GGC AGG GTC TTG ACC ACA ACC GGG ATC GCT 3071  
 5 1009 Ile Gly Asn Gln Ile Arg Gly Arg Val Leu Thr Thr Thr Gly Ile Ala 1014  
 3073 AAT GCG ATA GAC TAT GGC ACC AGT GCT GTG GGA GCC GCA CGA CGA GTT 3120  
 10 1025 Asn Ala Ile Asp Tyr Gly Thr Ser Ala Val Gly Ala Ala Arg Arg Val 1040  
 3121 TTT TCT TTG TAA 3132  
 1041 Phe Ser Leu End 1043

15

(2) INFORMATION FOR SEQ ID NO:61

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1043 amino acids  
 (B) TYPE: amino acid  
 20 (C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61 (Tccc peptide)

1 Met Ser Pro Ser Glu Thr Thr Leu Tyr Thr Gln Thr Pro Thr Val Ser 16  
 17 Val Leu Asp Asn Arg Gly Leu Ser Ile Arg Asp Ile Gly Phe His Arg 32  
 30 33 Ile Val Ile Gly Gly Asp Thr Asp Thr Arg Val Thr Arg His Gln Tyr 48  
 49 Asp Ala Arg Gly His Leu Asn Tyr Ser Ile Asp Pro Arg Leu Tyr Asp 64  
 35 65 Ala Lys Gln Ala Asp Asn Ser Val Lys Pro Asn Phe Val Trp Gln His 80  
 81 Asp Leu Ala Gly His Ala Leu Arg Thr Glu Ser Val Asp Ala Gly Arg 96  
 97 Thr Val Ala Leu Asn Asp Ile Glu Gly Arg Ser Val Met Thr Met Asn 112  
 40 113 Ala Thr Gly Val Arg Gln Thr Arg Arg Tyr Glu Gly Asn Thr Leu Pro 128  
 129 Gly Arg Leu Leu Ser Val Ser Glu Gln Val Phe Asn Gln Glu Ser Ala 144  
 45 145 Lys Val Thr Glu Arg Phe Ile Trp Ala Gly Asn Thr Thr Ser Glu Lys 160  
 161 Glu Tyr Asn Leu Ser Gly Leu Cys Ile Arg His Tyr Asp Thr Ala Gly 176  
 177 Val Thr Arg Leu Met Ser Gln Ser Leu Ala Gly Ala Met Leu Ser Gln 192  
 50 193 Ser His Gln Leu Leu Ala Glu Gly Gln Glu Ala Asn Trp Ser Gly Asp 208  
 209 Asp Glu Thr Val Trp Gln Gly Met Leu Ala Ser Glu Val Tyr Thr Thr 224  
 55 225 Gln Ser Thr Thr Asn Ala Ile Gly Ala Leu Leu Thr Gln Thr Asp Ala 240  
 241 Lys Gly Asn Ile Gln Arg Leu Ala Tyr Asp Ile Ala Gly Gln Leu Lys 256  
 257 Gly Ser Trp Leu Thr Val Lys Gly Gln Ser Glu Gln Val Ile Val Lys 272  
 60 273 Ser Leu Ser Trp Ser Ala Ala Gly His Lys Leu Arg Glu Glu His Gly 288  
 289 Asn Gly Val Val Thr Glu Tyr Ser Tyr Glu Pro Glu Thr Gln Arg Leu 304  
 65 305 Ile Gly Ile Thr Thr Arg Arg Ala Glu Gly Ser Gln Ser Gly Ala Arg 320

	321	Val	Leu	Gln	Asp	Leu	Arg	Tyr	Lys	Tyr	Asp	Pro	Val	Gly	Asn	Val	Ile	336
5	337	Ser	Ile	His	Asn	Asp	Ala	Glu	Ala	Thr	Arg	Phe	Trp	Arg	Asn	Gln	Lys	352
	353	Val	Glu	Pro	Glu	Asn	Arg	Tyr	Val	Tyr	Asp	Ser	Leu	Tyr	Gln	Leu	Met	368
	369	Ser	Ala	Thr	Gly	Arg	Glu	Met	Ala	Asn	Ile	Gly	Gln	Gln	Ser	Asn	Gln	384
10	385	Leu	Pro	Ser	Pro	Val	Ile	Pro	Val	Pro	Thr	Asp	Asp	Ser	Thr	Tyr	Thr	400
	401	Asn	Tyr	Leu	Arg	Thr	Tyr	Thr	Tyr	Asp	Arg	Gly	Gly	Asn	Leu	Val	Gln	416
15	417	Ile	Arg	His	Ser	Ser	Pro	Ala	Thr	Gln	Asn	Ser	Tyr	Thr	Thr	Asp	Ile	432
	433	Thr	Val	Ser	Ser	Arg	Ser	Asn	Arg	Ala	Val	Leu	Ser	Thr	Leu	Thr	Thr	448
	449	Asp	Pro	Thr	Arg	Val	Asp	Ala	Leu	Phe	Asp	Ser	Gly	Gly	His	Gln	Lys	464
20	465	Met	Leu	Ile	Pro	Gly	Gln	Asn	Leu	Asp	Trp	Asn	Ile	Arg	Gly	Glu	Leu	480
	481	Gln	Arg	Val	Thr	Pro	Val	Ser	Arg	Glu	Asn	Ser	Ser	Asp	Ser	Glu	Trp	496
25	497	Tyr	Arg	Tyr	Ser	Ser	Asp	Gly	Met	Arg	Leu	Leu	Lys	Val	Ser	Glu	Gln	512
	513	Gln	Thr	Gly	Asn	Ser	Thr	Gln	Val	Gln	Arg	Val	Thr	Tyr	Leu	Pro	Gly	528
	529	Leu	Glu	Leu	Arg	Thr	Thr	Gly	Val	Ala	Asp	Lys	Thr	Thr	Glu	Asp	Leu	544
30	545	Gln	Val	Ile	Thr	Val	Gly	Glu	Ala	Gly	Arg	Ala	Gln	Val	Arg	Val	Leu	560
	561	His	Trp	Glu	Ser	Gly	Lys	Pro	Thr	Asp	Ile	Asp	Asn	Asn	Gln	Val	Arg	576
35	577	Tyr	Ser	Tyr	Asp	Asn	Leu	Leu	Gly	Ser	Ser	Gln	Leu	Glu	Leu	Asp	Ser	592
	593	Glu	Gly	Gln	Ile	Leu	Ser	Gln	Glu	Glu	Tyr	Tyr	Pro	Tyr	Gly	Gly	Thr	608
	609	Ala	Ile	Trp	Ala	Ala	Arg	Asn	Gln	Thr	Glu	Ala	Ser	Tyr	Lys	Phe	Ile	624
40	625	Arg	Tyr	Ser	Gly	Lys	Glu	Arg	Asp	Ala	Thr	Gly	Leu	Tyr	Tyr	Tyr	Gly	640
	641	Tyr	Arg	Tyr	Tyr	Gln	Pro	Trp	Val	Gly	Arg	Trp	Leu	Ser	Ala	Asp	Pro	656
45	657	Ala	Gly	Thr	Val	Asp	Gly	Leu	Asn	Leu	Tyr	Arg	Met	Val	Arg	Asn	Asn	672
	673	Pro	Ile	Thr	Leu	Thr	Asp	His	Asp	Gly	Leu	Ala	Pro	Ser	Pro	Asn	Arg	688
	689	Asn	Arg	Asn	Thr	Phe	Trp	Phe	Ala	Ser	Phe	Leu	Phe	Arg	Lys	Pro	Asp	704
50	705	Glu	Gly	Met	Ser	Ala	Ser	Met	Arg	Arg	Gly	Gln	Lys	Ile	Gly	Arg	Ala	720
	721	Ile	Ala	Gly	Gly	Ile	Ala	Ile	Gly	Gly	Leu	Ala	Ala	Thr	Ile	Ala	Ala	736
55	737	Thr	Ala	Gly	Ala	Ala	Ile	Pro	Val	Ile	Leu	Gly	Val	Ala	Ala	Val	Gly	752
	753	Ala	Gly	Ile	Gly	Ala	Leu	Met	Gly	Tyr	Asn	Val	Gly	Ser	Leu	Leu	Glu	768
	769	Lys	Gly	Gly	Ala	Leu	Leu	Ala	Arg	Leu	Val	Gln	Gly	Lys	Ser	Thr	Leu	784
60	785	Val	Gln	Ser	Ala	Ala	Gly	Ala	Ala	Ala	Gly	Ala	Ser	Ser	Ala	Ala	Ala	800
	801	Tyr	Gly	Ala	Arg	Ala	Gln	Gly	Val	Gly	Val	Ala	Ser	Ala	Ala	Gly	Ala	816
	817	Val	Thr	Gly	Ala	Val	Gly	Ser	Trp	Ile	Asn	Asn	Ala	Asp	Arg	Gly	Ile	832
65	833	Gly	Gly	Ala	Ile	Gly	Ala	Gly	Ser	Ala	Val	Gly	Thr	Ile	Asp	Thr	Met	848

849 Leu Gly Thr Ala Ser Thr Leu Thr His Glu Val Gly Ala Ala Ala Gly 884  
 5 865 Gly Ala Ala Gly Gly Met Ile Thr Gly Thr Gln Gly Ser Thr Arg Ala 889  
 881 Gly Ile His Ala Gly Ile Gly Thr Tyr Tyr Gly Ser Trp Ile Gly Phe 896  
 897 Gly Leu Asp Val Ala Ser Asn Pro Ala Gly His Leu Ala Asn Tyr Ala 912  
 10 913 Val Gly Tyr Ala Ala Gly Leu Gly Ala Glu Met Ala Val Asn Arg Ile 923  
 929 Met Gly Gly Gly Phe Leu Ser Arg Leu Leu Gly Arg Val Val Ser Pro 944  
 15 945 Tyr Ala Ala Gly Leu Ala Arg Gln Leu Val His Phe Ser Val Ala Arg 960  
 961 Pro Val Phe Glu Pro Ile Phe Ser Val Leu Gly Gly Leu Val Gly Gly 976  
 977 Ile Gly Thr Gly Leu His Arg Val Met Gly Arg Glu Ser Trp Ile Ser 992  
 20 993 Arg Ala Leu Ser Ala Ala Gly Ser Gly Ile Asp His Val Ala Gly Met 1008  
 1009 Ile Gly Asn Gln Ile Arg Gly Arg Val Leu Thr Thr Thr Gly Ile Ala 1024  
 1025 Asn Ala Ile Asp Tyr Gly Thr Ser Ala Val Gly Ala Ala Arg Arg Val 1040  
 25 1041 Phe Ser Leu 1043

30

We claim:

1. A composition, comprising an effective amount of a  
*Photobhabdus* protein toxin that has functional activity against  
5 an insect.

2. The composition of Claim 1, wherein the *Photobhabdus*  
toxin is produced by a purified culture of *Photobhabdus*, a  
transgenic plant, Baculovirus, or heterologous microbial host.  
10

3. The composition of Claim 2, wherein the *Photobhabdus*  
toxin produced by a purified culture of *Photobhabdus luminescens*.

4. The composition of Claim 2, wherein the toxin is  
15 produced from a purified culture of *Photobhabdus luminescens*  
strain designated ATCC 55397.

5. The composition of Claim 2, wherein the toxin is  
produced by a purified culture of *Photobhabdus luminescens* strain  
20 designated W-14.

6. The composition of Claim 1, wherein the toxin is  
produced by a purified culture of *Photobhabdus* strain designated  
WX-1, WX-2, WX-3, WX-4, WX-5, WX6, WX-7, WX-8, WX-9, WX-10, WX-  
25 11, WX-12, WX-14, WX-15, H9, Hb, Hm, HP88, NC-1, W30, WIR, ATCC#  
43948, ATCC# 43949, ATCC# 43950, ATCC# 43951, or ATCC# 43952.

7. The composition of Claim 2, wherein the toxin is  
produced from a purified culture of *Photobhabdus luminescens*  
30 strain designated WX-1, WX-2, WX-3, WX-4, WX-5, WX-6, WX-7, WX-8,  
WX-9, WX-10, WX-11, WX-12, WX-14, WX-15, H9, Hb, Hm, HP88, NC-1,  
W30, WIR, ATCC# 43948, ATCC# 43949, ATCC# 43950, ATCC# 43951, or  
ATCC# 43952.

8. The composition of Claim 1, wherein the toxin is  
35 represented by amino acid sequence is SEQ ID NO:12.

9. The composition of Claim 6, wherein the composition is a  
mixture of one or more toxins produced from purified cultures of  
40 *Photobhabdus*.

10. The composition of Claim 1 or 6, wherein the insect is of the order *Lepidoptera*, *Coleoptera*, *Hymenoptera*, *Diptera*, *Dictyoptera*, *Acarina* or *Homoptera*.

5

11. The composition of Claim 1 or 6, wherein the insect species is from order *Coleoptera* and is Southern Corn Rootworm, Western Corn Rootworm, Colorado Potato Beetle, Mealworm, Boll Weevil or Turf Grub.

10

12. The composition of Claim 1 or 6, wherein the insect species is from order *Lepidoptera* and is Beet Armyworm, Black Cutworm, Cabbage Looper, Codling Moth, Corn Earworm, European Corn Borer, Tobacco Hornworm, or Tobacco Budworm.

15

13. The composition of Claim 1 or 6, wherein the toxin is formulated as a sprayable insecticide.

14. The composition of Claim 1 or Claim 6, wherein the toxin is formulated as a bait matrix and delivered in an above ground or below ground bait station.

15. A method of controlling an insect, comprising orally delivering to an insect an effective amount of a protein toxin that has functional activity against an insect, wherein the protein is produced by a purified bacterial culture of the genus *Photobacterium*.

16. The method of Claim 15, wherein the bacterium is a purified culture of *Photobacterium luminescens*.

17. The method of Claim 15, wherein the toxin is produced from a purified culture of *Photobacterium luminescens* strain designated ATCC 55397.

35

18. The method of Claim 16, wherein the toxin is produced from a purified culture of *Photobacterium luminescens* strain designated W-14.



19. The method of Claim 15, wherein the toxin is produced from a purified culture of *Photobhabdus* strains designated WX-1, WX-2, WX-3, WX-4, WX-5, WX-6, WX-7, WX-8, WX-9, WX-10, WX-11, WX-12, WX-14, WX-15, H9, Hb, Hm, HP88, NC-1, W30, WIR, ATCC# 43948, ATCC# 43949, ATCC# ATCC# 43950, ATCC# 43951, or ATCC# 43952.

20. The method of Claim 15, wherein the toxin is produced from a purified culture of *Photobhabdus luminescens* strains designated WX-1, WX-2, WX-3, WX-4, WX-5, WX-6, WX-7, WX-8, WX-9, WX-10, WX-11, WX-12, WX-14, WX-15, H9, Hb, Hm, HP88, NC-1, W30, WIR, ATCC# 43948, ATCC# 43949, ATCC# ATCC# 43950, ATCC# 43951, or ATCC# 43952.

21. The method of Claim 19, wherein a mixture of one or more toxins is produced from a purified culture of *Photobhabdus* and said toxins are orally delivered to an insect.

22. The method of Claim 15, wherein the toxin is produced by a prokaryotic host transformed with a gene encoding the toxin.

23. The method of Claim 15, wherein the toxin is produced by a eukaryotic host transformed with a gene encoding the toxin.

24. The method of Claim 23, wherein the eukaryotic host is baculovirus.

25. The method of Claim 15 or 19, wherein the insect is of the order *Lepidoptera*, *Coleoptera*, *Hymenoptera*, *Diptera*, *Dictyoptera*, *Acarina* or *Homoptera*.

26. The method of Claim 15 or 19, wherein the insect species is from order *Coleoptera* and is Southern Corn Rootworm, Western Corn Rootworm, Colorado Potato Beetle, Mealworm, Boll Weevil or Turf Grub.

27. The method of Claim 15 or 19, wherein the insect species is from order *Lepidoptera* and is Beet Armyworm, Black Cutworm, Cabbage Looper, Codling Moth, Corn Earworm, European Corn Borer, Tobacco Hornworm, or Tobacco Budworm.

28. The method of Claim 15 or 19, wherein the toxin is formulated as a sprayable insecticide.

29. The method of Claim 15 or Claim 19, wherein the toxin is formulated as a bait matrix and delivered in an above ground or below ground bait station.

30. A method of isolating a gene coding for a protein subunit, comprising the steps of: constructing at least one RNA or DNA oligonucleotide molecule that corresponds to at least a part of a DNA coding region of an amino acid sequence selected from a group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, and SEQ ID NO:43, wherein the nucleotide molecule is used to isolate genetic material from *Photorhabdus* or *Photorhabdus luminescens*.

31. A method for expressing a protein produced by a purified bacterial culture of the genus *Photorhabdus* in a prokaryotic or eukaryotic host in an effective amount so that the protein has functional activity against an insect, wherein the method comprises: constructing a chimeric DNA construct having 5' to 3' a promoter, a DNA sequence encoding a protein, a transcription terminator, and then transferring the chimeric DNA construct into the host.

32. The method of Claim 31, wherein the protein has functional activity against insects selected from a group consisting of *Coleoptera*, *Lepidoptera*, *Diptera*, *Homoptera*, *Hymenoptera*, *Dictyoptera*, and *Acarina*.

33. The method of Claim 31, wherein the protein encoded by the DNA sequence has an N-terminal amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:14, SEQ

ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, and SEQ ID NO:43.

5

34. The method of Claim 31, wherein the protein encoded by the DNA sequence includes the amino acid sequence selected from the group consisting of SEQ ID NO:12, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59 and SEQ ID NO:61.

35. A chimeric DNA construct, adapted for expression in a prokaryotic or eukaryotic host comprising, 5' to 3' a transcriptional promoter active in the host; a DNA sequence encoding a *Photobacterium* protein that has functional activity against an insect; and a transcriptional terminator.

36. A chimeric DNA construct of Claim 35, wherein the protein encoded by the DNA sequence has an N-terminal amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, and SEQ ID NO:43.

37. The chimeric DNA construct of Claim 35, wherein the protein encoded by the DNA sequence has an amino acid sequence selected from the group consisting of SEQ ID NO:12, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, and SEQ ID NO:61.

35

38. The chimeric DNA construct of Claim 35, wherein the DNA sequence encoding the *Photobacterium luminescens* protein is selected from the group comprising SEQ ID NO:11, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID

NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54,  
SEQ ID NO:56, SEQ ID NO: 58, and SEQ ID NO:60.

39. The chimeric DNA construct of Claim 35, wherein the  
5 host is baculovirus.

40. An isolated and substantially purified preparation  
comprising, a DNA molecule capable of encoding an effective  
amount of a protein that is produced by a bacterium of the genus  
10 *Photorhabdus* and that has functional activity against an insect.

41. The preparation of Claim 40, wherein the bacterium is  
*Photorhabdus luminescens*.

15 42. A purified preparation comprising, a protein produced  
by *Photorhabdus* or *Photorhabdus luminescens* having an N-terminal  
amino acid sequence selected from the group consisting of SEQ ID  
NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID  
NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID  
20 NO: 13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17,  
SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID  
NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:38, SEQ ID NO:39,  
SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, and SEQ ID NO:43.

25 43. A purified protein preparation comprising, a protein  
that has an N-terminal amino acid sequence selected from the  
group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID  
NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID  
NO:9, and SEQ ID NO:10, SEQ ID NO: 13, SEQ ID NO:14, SEQ ID NO:15,  
30 SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID  
NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24,  
SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID  
NO:42, and SEQ ID NO:43.

35 44. A purified protein preparation comprising, a protein  
selected from the group of SEQ ID NO:12, SEQ ID NO:26, SEQ ID  
NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:35,  
SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID  
NO:55, SEQ ID NO:57, SEQ ID NO:59, and SEQ ID NO:61.

40

45. A purified DNA preparation comprising, a DNA sequence selected from the group consisting of SEQ ID NO:11, SEQ ID NO:15, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, 5 SEQ ID NO:56, SEQ ID NO:58 and SEQ ID NO:60, wherein the DNA sequence is isolated from its native host.

46. A purified protein preparation comprising, a *Photorhabdus luminescens* protein with at least one subunit having 10 an approximate molecular weight between 18 kDa to about 230 kDa; between about 160 kDa to about 230 kDa; 100 kDa to 160 kDa; about 80 kDa to about 100 kDa; or about 50 kDa to about 80 kDa.

47. A purified protein preparation comprising, a 15 *Photorhabdus luminescens* protein with at least one subunit having an approximate molecular weight of about 280 kDa.

48. A substantially pure microorganism culture comprising, ATCC 55397. 20

49. The culture of Claim 48, wherein the culture is a derivative of ATCC 55397 that produces a protein toxin that has functional activity against an insect.

50. A substantially pure microorganism culture comprising, H9. 25

51. A substantially pure microorganism culture comprising, Hb. 30

52. A substantially pure microorganism culture comprising, Hm.

53. A substantially pure microorganism culture comprising, HP88. 35

54. A substantially pure microorganism culture comprising, NC-1.

55. A substantially pure microorganism culture comprising. 40

W30.

56. A substantially pure microorganism culture comprising,  
WIR.

5

57. A transgenic plant comprising in its genome, a chimeric artificial gene construction imbuing the plant with an ability to express an effective amount of a *Photorhabdus* protein that has functional activity against an insect.

10

58. The transgenic plant of Claim 57, wherein the plant is transformed using acceleration of genetic material coated onto microparticles directly into cells, *Agrobacteria*, whiskers, or electroporation techniques

15

59. The transgenic plant of Claim 57, wherein the selectable marker is selected from the group consisting of kanamycin, neomycin, glyphosate, hygromycin, methotrexate, phosphinothricin (bialophos), chlorosulfuron, bromoxynil, dalapon and the like.

20

60. The transgenic plant of Claim 57, wherein the promoter is selected from the group consisting of octopine synthase, nopaline synthase, mannopine synthase, 35S, 19S, ribulose-1,6-bisphosphate (RUBP) carboxylase small subunit (ssu), beta-conglycinin, phaseolin, alcohol dehydrogenase (ADH), heat-shock, ubiquitin, zein, oleosin, napin, or acyl carrier protein (ACP).

25

61. The transgenic plant of Claim 57, wherein embryogenic tissue, callus tissue type I or II, hypocotyl, meristem, or plant tissue during dedifferentiation is used in preparing the transgenic plant.

30

62. The transgenic plant of Claim 57, wherein the chimeric gene is a DNA sequence which encodes a *Photorhabdus* protein that has functional activity against an insect and at least one codon of the gene has been modified so that the codon is a plant preferred codon.

35

63. A method of controlling an insect comprising orally delivering to an insect an effective amount of a protein toxin, wherein the protein is produced by a transgenic plant, which said insect feeds.

5

64. A composition of matter, comprising a purified DNA sequence from a purified bacterial culture from the genus *Photorhabdus*.

1 ATG CAG GAT TGT CCG GAA GTA TOG ATT ACA ACG CTG TCA CTT CCC AAA GGT GGC GGT  
 TAC GTC CTA ACA GGC CTT CAT AGC TAA TGT TGC GAC AGT GAA GGG TTT CCA CCG CCA  
 1 Met Gln Asp Cys Pro Glu Val Ser Ile Thr Thr Leu Ser Leu Pro Lys Gly Gly Gly  
 P2Psh  
 58 GCT ATC AAT GGC ATG GGA GAA GCA CTG AAT GCT GGC GGC OCT GAT GGA ATG GGC TOC  
 CGA TAG TTA CCG TAC OCT CTT CGT GAC TTA CGA CCG CCG GGA CTA OCT TAC CCG AGG  
 20 Ala Ile Asn Gly Met Gly Glu Ala Leu Asn Ala Ala Gly Pro Asp Gly Met Ala Ser  
 115 CTA TCT CTG CCA TTA OCC CTT TOG AOC GGC AGA GGG ACG GCT CCT GGA TTA TOG CTG  
 GAT AGA GAC GGT AAT GGG GAA AGC TGG CCG TCT OCC TGC CGA GGA OCT NAT AGC GAC  
 39 Leu Ser Leu Pro Leu Pro Leu Ser Thr Gly Arg Gly Thr Ala Pro Gly Leu Ser Leu  
 172 ATT TAC AGC AAC AGT GCA GGT AAT GGG OCT TTC GGC ATC GGC TGG CAA TGC GGT GTT  
 TAA ATG TCG TTG TCA CGT CCA TTA OCC GGA AAG CCG TAG CCG AOC GTT ACG CCA CAA  
 58 Ile Tyr Ser Asn Ser Ala Gly Asn Gly Pro Phe Gly Ile Gly Trp Gln Cys Gly Val  
 229 ATG TOC ATT AGC CGA CCG ACC CAA CAT GGC CTT CAA CAT TGA CGA CGT  
 TAC AGG TAA TCG GCT GCG TGG GTT GTA CCG GAA GTT GTA ACT GCT GCA  
 77 Met Ser Ile Ser Arg Arg Thr Gln His Gly Leu Gln His ... Arg Arg  
 P2.3.5R

FIG. 1



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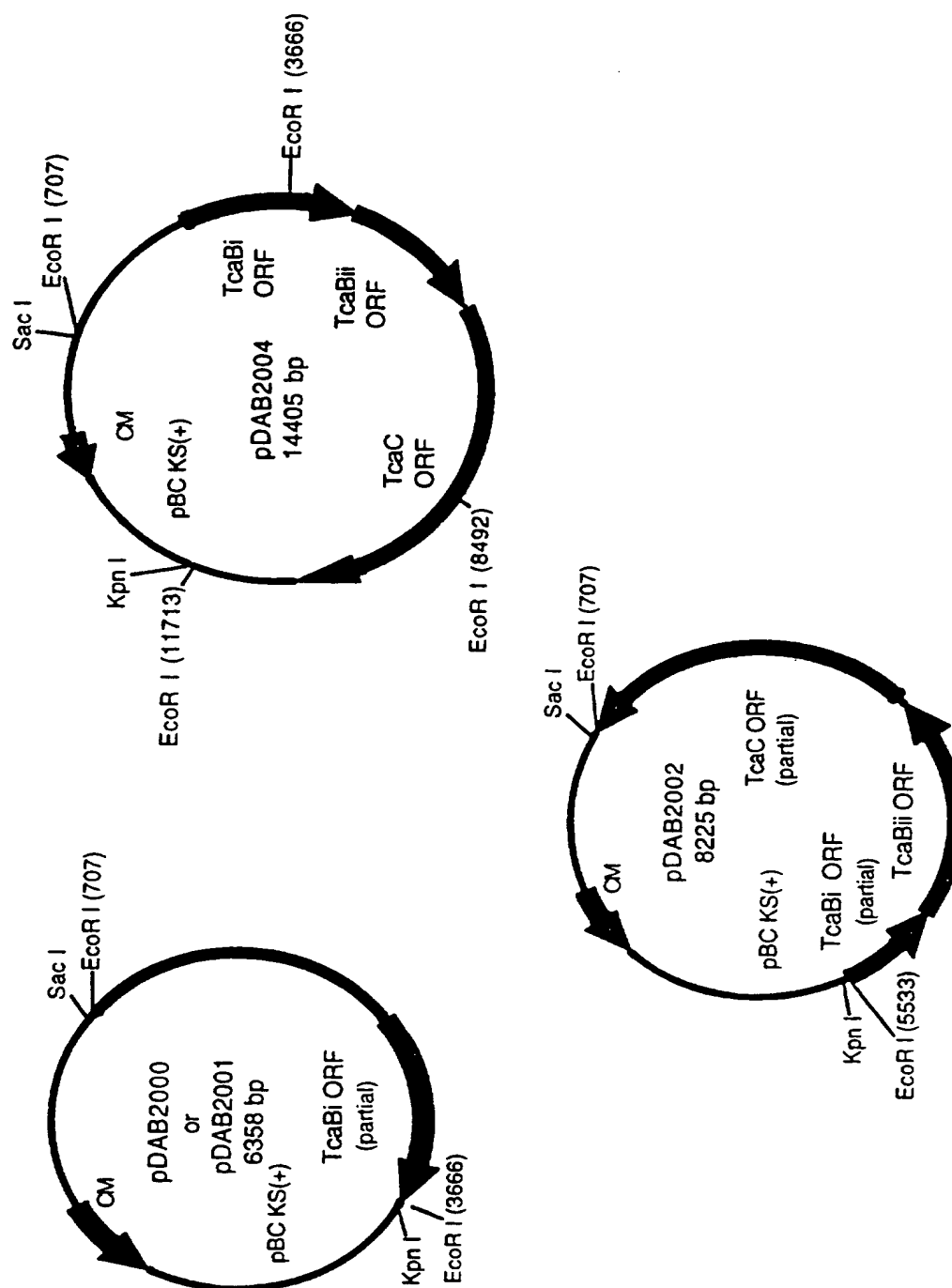


FIG. 2

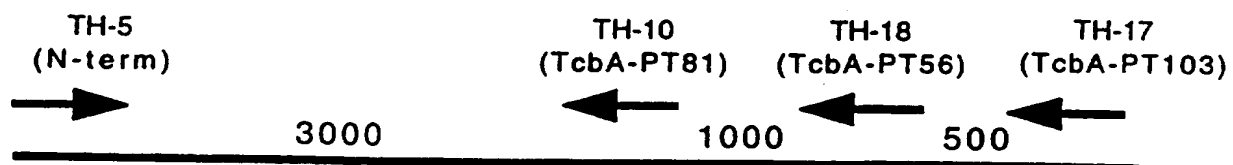


FIG. 3

TcbA	1740	1750	1760	1770	1780
	SSAQALKNDS	EPMDFSGANA	LYFWELFYTT	PMMMAHRLLO	EQNFDAANHW
		n			
		450	460	470	480
TcaBi	gS	nPvDFSGpyg	iYlWEIfFhi	PflvtvRmqt	EQryedAdtW>
	^^	^^^^^^^v	^^^^^^^	^^^^v^v	^^-^^-^^v
TcbA	1790	1800	1810	1820	1830
	FRYVWSPSGY	IVDGKIAIYH	WNVRLPLEEDT	SWNAQQLDST	DPDAVAQDDP
	rdangql				
	490	510	520	530	
TcaBi	yKyiFrSaGY	ImDGskprY-	WNVmPLqlDT	aWdttQpatT	DPDviAmaDP>
	^^^^-^^^	^^^^-v^v	^^-^^^v^^	^^^^v^v-^^	^^-^^v-^^
TcbA	1840	1850	1860	1870	1880
	MHYKVATFMA	TLDLLMARGD	AAYRQLERDT	LAEAKMWTQ	ALNLLGDEPO
	540	550	560	570	580
TcaBi	MHYKlAiFlh	TLDLLiARGD	sAYRQLERDT	LvEAKMyYiQ	AqqLLGprPd>
	^^^^-^^v	^^^^-^^v	^^^^-^^^	^^-^^-^^	^^-^^-^^v^^
TcbA	1890	1900	1910	1920	1930
	VMLSTIWANP	TLGNAASKTT	QQVRQQVLTO	LRLNSRVKTP	LLGTANSLTA
		600			
TcaBi	ihhtnTWpNP	TLsk>			
	^^v-^^-^^	^^^			
TcbA	1940	1950	1960	1970	1980
	LFLPQENSKL	KGyWRTLAQR	MFNLRHNLST	DGQPLSLPLY	AKPADPKALL
	20	30	40	50	60
TcaBii	_FLPpyNdVL	lGYWdkLeIR	lyNLRHNLST	DGQPLnLPLY	AtPvDPKtLq>
	^^-v^v	v^^v-^^v	^^^^-^^^	^^^^-^^^	^^-^^-^^v
TcbA	1990	2000	2010	2020	2030
	SAAVSASQGG	ADLPKAPLTI	HRFPQMLEGA	RGLVNQLIQF	GSSLLGYSER
		gw			
	70	80	90	100	110
TcaBii	rqqagggdgtG	sspaggggsv	qRyPlIvErA	RsaVslLtQF	GnSLqttilEh>
	^^-^^-v-	^^-v^v-^^	^^^^v^^v	^^v^v-^^	^^^v-vv^^
TcbA	2040	2050	2060	2070	2080
	QDAEAMSQLL	QTQASELILT	SIRMQDNQLA	ELDSEKTALQ	VSLAGVQQRF
	120	130	140	150	160
TcaBii	QDnEkMtill	QTQgeailkh	qhdiQqNnLk	gLqhsLTALQ	aSrdGdtlRq>
	^^-v^v	^^-^^-^^v	vvv^^^^v	^^v-v^^	^^v-^^v^v

FIG. 4A

TcbA	2090	2100	2110	2120	2130
	DSYSQLYEEN	INAGEQRALA	LRSESAIESQ	GAQISRMAGA	GVDMAFNIFG
TcaBii	170	180	190	200	210
	khYSdLingg	lsAaEiagLt	LRStamI-tn	Gvatglliag	GinavPNvFG>
	-v^^^v^--	^^^v^v^^	^^^v^v^^	^^^v^v^^	^^^v^v^^
TcbA	2140	2150	2160	2170	2180
	LADGGMHYGA	IAYAIADGIE	LSASAKMVDA	EKVAQSEIYR	RRRQEWKIQR
TcaBii	220	230	240	250	260
	LAnGGsewGA	pligsgqatq	vgAgigdqsa	gisevtagYq	RRqeEWalQR>
	^^^v^v^^	vvv^v^v^^	^^^v^v^^	-vv-v^v^^	^^^v^v^^
TcbA	2190	2200	2210	2220	2230
	DNAQAEINQL	NAQLESLSIR	REAAEMQKEY	LKTQQAQAQA	QLTFLRSKFS
TcaBii	270	280	290	300	310
	DiAdnEItQL	dAQiqSLqeq	itmAqkQitl	seTeQAnAQA	iydlqttrFt>
	^v^v^v^^	^^^v^v^^	v-v^v^v^^	v-^^^v^v^^	vv-^v^v^^
TcbA	2240	2250	2260	2270	2280
	NQALYSWLRG	RLSGIYFQFY	DLAVSRCLMA	EQSYQWEAND	NSISFVKPGA
TcaBii	320	330	340	1	360
	gQALYnWmaG	RLSalyYQmY	DstlpicLqp	kaalvqEgek	eSdSlfqvpv>
	-^^^v^v^^	^^^v^v^^	^v^v^v^^	--vvv^v^^	^^v^v^v^^
TcbA	2290	2300	2310	2320	2330
	WQGTYAGLLC	GEALIQNLAQ	MEEAYLKWES	RALEVERTVS	LAVVYDSLEG
TcaBii	370	380	390	400	410
	WndlwgGLLa	GEgLsseLqk	ldaiwLargg	igLEaiRTVS	Ldtlfgt--G>
	^^v--^^v	^^^v^v^^	^^-v-^v^^	v^^^v^v^^	^^-^^^v^v^^
TcbA	2340	2350	2360	2370	2380
	NDRFNLAEQI	PALLDKGEGT	AGTKKNGLSL	ANAILSASVK	LSDLKLGTDY
TcaBii	420	430	440	450	
	----tLsEnI	nkvlLn-GETv	spsggvtLaL	tgdlfqAtld	LSqLgLdnsY>
	-^^^v^v^^	vv^^^v^v^^	^v^v^v^^	^^^v^v^^	^^^v^v^^
TcbA	2390	2400	2410	2420	2430
	PDSIVGSNKV	RRIKQISVSL	PALVGPYQDV	QAMLSYGGST	QLPKGCSALA
TcaBii	460	470	480	490	500
	-n--lGneKk	RRIKrIaVtL	PtLlGPYQDl	eAtLvmGaee	aLshGvndgg>
	^ ^^^v^v^^	^^^v^v^^	^^^v^v^^	^^v^v^v^^	^^^v^v^^
TcbA	2440	2450	2460	2470	
	VSHGTNDSGQ	FQLDFNDGKY	LPFEGIALDD	QGTNLNLQFPN	
TcaBii	510	520	530		
	rfvtdfndsr	F-LpF-eGrd	attgtleLn>		
	vvv--v^v^^	^v^v^v^^	v-v--^v^v^^		

FIG. 4B

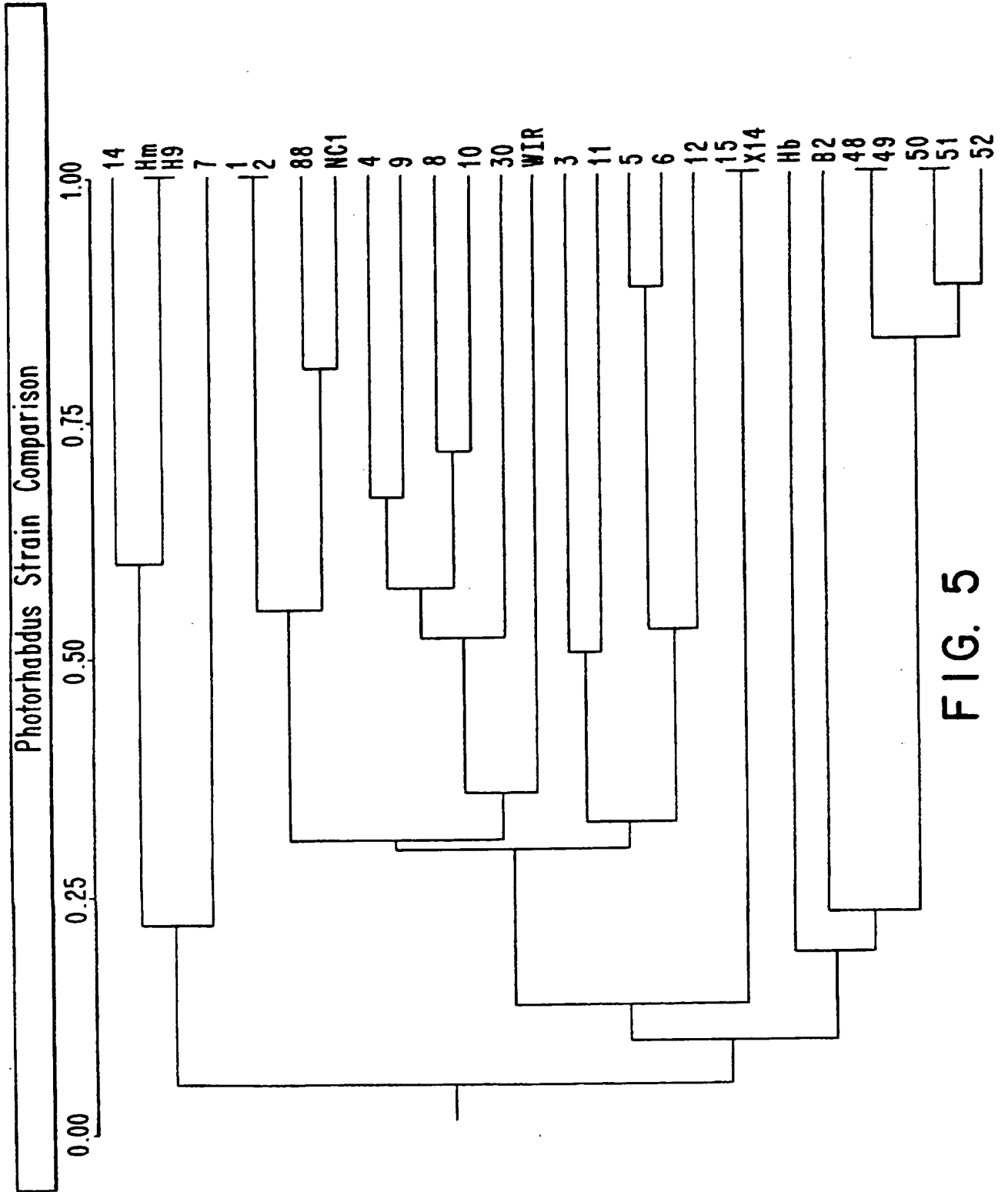


FIG. 5

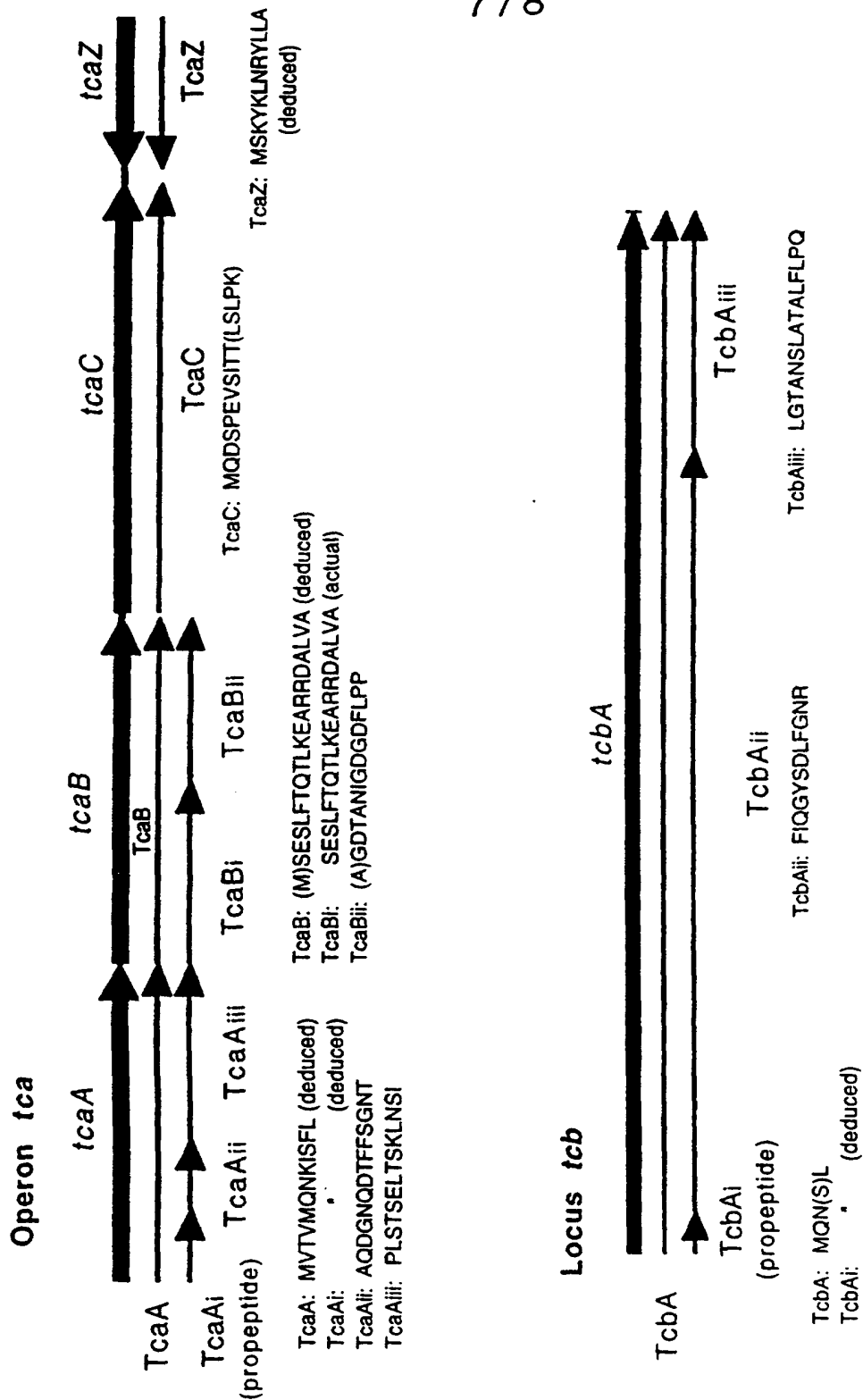


FIG. 6A

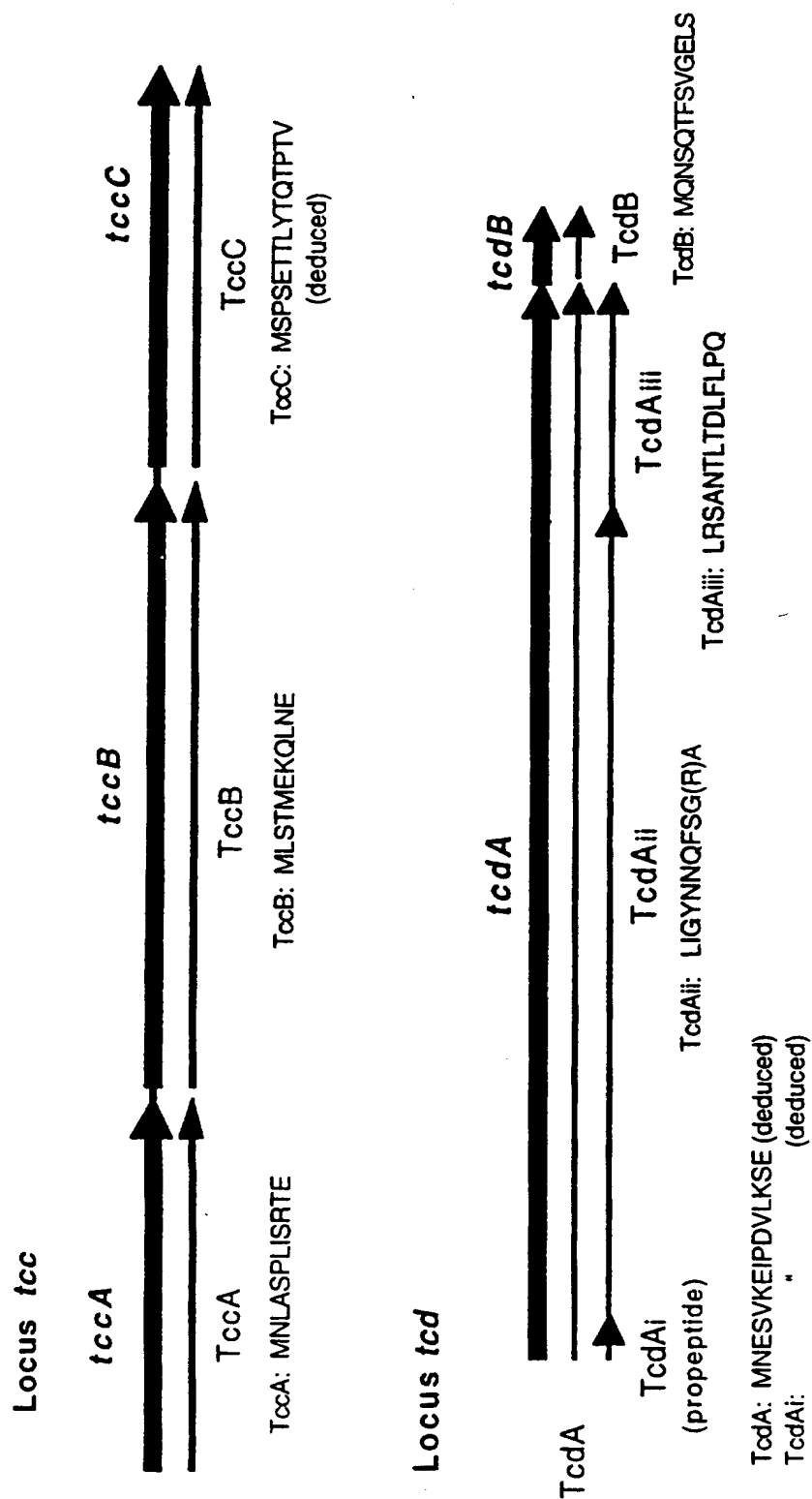


FIG. 6B

## INTERNATIONAL SEARCH REPORT

International application N.  
PCT/US96/18003

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : Please See Extra Sheet.

US CL : 536/23.7, 24.1; 435/172.3, 240.4, 320.1; 800/205; 47/58

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.7, 24.1; 435/172.3, 240.4, 320.1; 800/205; 47/58

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CABA, CAPLUS, MEDLINE, GENBANK, BIOSIS

search terms: photorhabdus, xenorhabdus, luminescens, insecticide, nematode, lepidoptera

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CLARKE et al. Virulence Mechanisms of <i>Photorhabdus</i> sp. Strain K122 toward Wax Moth Larvae. Journal of Invertebrate Pathology. 1995, Vol. 66, pages 149-155, see entire document.	1-64
Y	US 5,039,523 A (PAYNE ET AL.) 13 August 1991, columns 1-10.	1-64
Y	US 5,254,799 A (DE GREVE ET AL.) 19 October 1993, columns 1-14.	1-64

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A*	document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means		
*P* document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

23 DECEMBER 1996

Date of mailing of the international search report

28 JAN 1997

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
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# INTERNATIONAL SEARCH REPORT

International application N .  
PCT/US96/18003

## A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

C12N 5/14, 15/00, 15/05, 15/09, 15/29, 15/31, 15/64, 15/82; A01G 13/00; A01H 1/00

